THE INFLUENCE OF LYOPHILIC COLLOIDS ON THE FORMATION OF A NEW PHASE, AND THE COMBINATION OF GELATIN WITH SILVER IONS.

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Thesis for the degree of Doctor of Philosophy.

University of Edinburgh. May 1941.

C O N T E N T S.

I N T R O D U C T I O N.

It is well-known that crystallisation, or precipitation, or, in general, the formation of a new phase from a supersaturated solution may be markedly delayed by the presence of even small quantities of foreign material.[1] In particular, it has been observed that lyophilic colloids, such as gelatin and agar, often retard the separation of the solid phase from solutions containing excess of ions which would normally combine to form a sparingly soluble salt. The case which has probably received most attention is that of the influence of gelatin upon the precipitation of silver chromate, which is of special interest in connection with the Liesegang phenomenon.

Williams and Mackenzie[2] appear to have been the first to show definitely that in the presence of gelatin, a mixture of solutions of silver nitrate and potassium chromate may remain quite clear and yellow for at least 72 hours, although the concentrations of the salts are much in excess of those sufficient to produce an immediate precipitation of silver chromate (red) in the

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(1) See, for example, Freundlich, "Kapillarchemie", Vol. I (1930) 476.
(2) Williams and Mackenzie, J.C.S., 117 (1920) 844.

absence of the protein. Subsequent investigations[1][2][3] have established that in the yellow systems some of the silver and chromate ions are probably combined with the gelatin, while the rest are free and form a supersaturated solution of silver chromate. Thus the gelatin appears to oppose the precipitation in two ways, (a) by decreasing the concentrations of simple silver and chromate ions, and (b) by inhibiting the formation of stable crystal nuclei of silver chromate. It will be convenient to designate the second of these effects as the "inhibitive action" of the gelatin.

One of the objects of the present investigation was to ascertain the relation between the concentration of the gelatin and the extent of the inhibitive action, a knowledge of which is essential for the proper understanding of the behaviour of the gelatin. Bolam and Donaldson[4] found that the degree of supersaturation (defined as actual concentration of free silver ions/ concentration of silver ions in equilibrium with the free chromate ions present) appeared to be roughly constant for 0.32 to 1.69 per cent. solutions of the gelatin employed. It was therefore deemed probable that the inhibitive action at first increases with increase in the gelatin concentration, but reaches its

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(1) Bolam and Mackenzie, Trans. Faraday Soc., 23 (1926) 151 and 162.
(2) Bolam and Donaldson, Trans. Faraday Soc., 29 (1933) 864.
(3) Desai and Nabar, Trans. Faraday Soc., 28 (1932) 449.
(4) Bolam and Donaldson, loc. cit.

maximum value at some low concentration of the protein, so that further addition of gelatin has no effect. This has now been definitely established.

In the case of the systems examined by previous workers, the concentrations of free silver ion, free chromate ion and gelatin usually all varied together, so that it was difficult to measure the degree of supersaturation and to study quantitatively the influence of various factors upon the extent of the inhibitive action of the gelatin. The present work has shown that it is possible to obtain in a simple manner systems in which the free chromate ion concentration remains constant while other factors are varied. These simplified experimental conditions have been utilised to investigate more exactly the inhibitive action of the gelatin, particularly as influenced by (a) the concentration of the gelatin, (b) the pH and (c) the previous treatment of the gelatin.

The suggestion that in mixed solutions of silver nitrate and gelatin a portion of the silver ions is combined with the protein, appears to have been first put forward by Pauli and Matula.[1] These workers determined the activity of the silver ion electrometrically by means of the silver electrode, and found that it appeared to be less in the presence than in the absence of gelatin. Pauli and Matula's observation

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(1) Pauli and Matula, Biochem. Zeitschr., 80 (1917)187.

has been fully confirmed by others,[1][2][3][4][5][6] but a decrease in the activity is in itself insufficient to establish that the gelatin forms a compound with the silver ion, since it is possible that the activity may be reduced by interaction (of the Debye-Hückel type) with the polyvalent gelatin ions. There are, however, other facts which support the view that combination does occur. Northrop and Kunitz[7] have reported that when equilibrium is reached between a solution of iso-electric gelatin and a solution of silver nitrate which are separated by a membrane permeable only to the silver salt, then (a) the concentration of silver nitrate is considerably greater on the gelatin side of the membrane, and (b) a membrane potential is set up, the sign of which indicates that the protein is positively charged. It is difficult to account for these results on any assumption other than that of combination of the gelatin with silver ions. Bolam and Mackenzie[2] found that the conductivity of a gelatin solution containing silver nitrate was very much less than that of one containing potassium nitrate under the same conditions of concentration, which again is strongly indicative of

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(1) Audubert, Comptes Rendus, 12 (1923) 838.
(2) Bolam and Mackenzie, loc. cit.
(3) Bolam and Donaldson, loc. cit.
(4) Kruyt and Boelman, Koll. Beih., 35 (1932) 165.
(5) Carroll and Hubbard, U.S. Bureau of Stand ards, J. Res., 7 (1931) 811. A full account of this paper is given in Schmidt, "The Chemistry of the Amino Acids and Proteins" (1938) 695.
(6) Goigner and Pauli, Biochem. Z., 235 (1931) 271.
(7) Northrop and Kunitz., J. Gen. Physiol., 11 (1928) 481.

combination. The well known experiments of Loeb,[1] in which granules of gelatin, after adjustment of the pH, were immersed in solutions of silver nitrate, and then washed with water and examined for the presence of silver, appear to be inconclusive, since the same results might be produced irrespective of whether the silver was combined with the gelatin, or simply held electrostatically as the cation of a strongly ionised gelatin salt.

The most comprehensive investigation of the interaction of gelatin with silver ions is that of Carroll and Hubbard,[2] who have studied the influence of the pH upon the activity of the silver ion, in solutions of silver nitrate containing gelatin, by means of the silver electrode. Carroll and Hubbard conclude that combination of the gelatin with silver ion increases with increase in pH.[3] According to their derived data, when the concentration of free silver ion is kept constant at 0.35 milli-equiv. (or more) per litre, the amount of silver combined with unit weight of gelatin varies with the pH in the following manner (pH values approximate) - from pH = 2 to pH = 4, small increase in in combined silver; pH = 4-6, large increase; pH = 6-7, small increase; pH = 7-9, possibly large increase.

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(1) Loeb, "Proteins and the Theory of Colloidal Behaviour" (1922).
(2) Carroll and Hubbard, loc. cit.
(3) It may be noted that Loeb (loc. cit.) observed that silver was much more readily retained by gelatin on the alkaline side of the iso-electric point than on the acid side.

Carroll and Hubbard suggest that the effect produced by increase in the pH is due to the formation of amino groups at the peptide linkages, and the subsequent formation of silver ion-amino complexes. Thus:-

$$R - CO - NH - R' + NaOH \longrightarrow R\text{-}COONa + R' - NH_2$$

$$R' - NH_2 + Ag^+ \longrightarrow R - NH_2Ag^+$$

It is obvious that the same result is more simply obtained on the basis of the zwitterion concept, as suggested by Schmidt.(1) Thus:-

$$R - NH_3^+ + OH^- \longrightarrow R - NH_2 + H_2O$$

$$R - NH_2 + Ag^+ \longrightarrow R - NH_2Ag^+$$

Carroll and Hubbard state that in alkaline solutions of gelatin the silver electrode is easily poisoned, so that their data became uncertain at pH values greater than 7.0.

In the present work the question of the combination of gelatin with silver ions has been further investigated by methods other than those previously employed. It was found that under the simplified conditions the precipitation experiments not only furnished more exact information with regard to the inhibitive action, but also provided a new and direct method of measuring the amount of combination between the protein and the silver. Parallel determinations

(1) Schmidt, "The Chemistry of the Amino Acids and Proteins" (1938) 696.

with the silver electrode and the precipitation technique gave results which closely agreed when the gelatin solution was neutral, but diverged very considerably at higher pH values, thus showing that the electrometric method is unreliable under these conditions. Advantage was taken of the precipitation method to determine the amount of combination over the pH range 7.0 to 10.5, in the case of both ordinary and deaminated gelatin.

In addition to estimations of the amount of combination, the influence of silver nitrate upon the electro-kinetic behaviour of ordinary and deaminated gelatin has been examined, in order to obtain information with regard to the nature of the combination. The electrophoresis of solid particles suspended in the gelatin solutions was first investigated, since valuable results have been obtained by this means in other connections.(1) The electrophoretic experiments, however, gave anomalous results, and eventually a study was made of the influence of silver nitrate upon the rate of electro-endosmotic flow through gelatin gels.

In the sequel an account is given of the experiments carried out in the investigation, followed by a discussion of the results obtained.

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(1) See, for example, Abramson, Gorin and Moyer, Chem. Rev., 24 (1939) 345. Abramson, Trans. Faraday Soc., 36 (1940) 5.

E X P E R I M E N T A L.

DESCRIPTION OF GELATIN.

Stock Gelatin. The gelatin (D) used in this research was supplied by Messrs Cox Ltd. of Edinburgh, being a sample of their highest grade of material and prepared in the same manner as the gelatin (A) used by Williams and Mackenzie,[1] the gelatin (B) used by Bolam and Mackenzie[2] and the gelatin (C) employed by Bolam and Donaldson.[3] When dried to constant weight at 105°, it was found to contain 16.79% moisture, and on ignition the dried material gave 0.82% ash. It may be added that gelatin (A) gave 17.62% moisture and 1.20% ash; gelatin (B), 17.65% moisture and 1.00% ash, and gelatin (C), 15.20% moisture and 0.96% ash.

Hydrolysed Gelatin. An amount of stock gelatin was weighed out, dissolved in 100 mls. of water, and the solution either heated on a water-bath under reflux for one hour or boiled under reflux for two hours.[4]

Purified Gelatin. For certain sections of the work pure de-ashed gelatin was used. It was prepared as follows: 200 gms. of gelatin (D) were stirred with 200 mls. of 0.01 N. sodium hydroxide solution for one

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(1) Williams and Mackenzie, J.C.S., 117 (1920) 844.
(2) Bolam and Mackenzie, Trans. Faraday Soc., 22 (1926) 151.
(3) Bolam and Donaldson, Trans. Faraday Soc., 29 (1933) 864.
(4) Pyrex flask and condenser used.

hour. The gelatin was next washed three times by stirring with distilled water for half-hour periods. It was then treated for one hour with 0.01 N. acetic acid, washed with three changes of water, again treated with 0.01 N. acetic acid, and finally washed with five changes of water. The gelatin was then filtered through muslin, perfused with absolute alcohol followed by ether, and allowed to dry by exposure to the atmosphere for 4 days. When dry, the product was light yellow, differing very little in appearance from gelatin D.

Analysis for moisture and ash gave the following results:-

% moisture	(1) 21.96	% ash	(1) 0.09
	(2) 22.07		(2) 0.07
Mean =	22.02%	Mean =	0.08%

Deaminated Gelatin. According to Hitchcock[1] deaminated gelatin prepared either with or without heating contains no amino nitrogen detectable by either the Van Slyke or the formol titration method. As heating hydrolyses the gelatin, it was avoided in this instance. The method of preparation adopted was as follows:- To 100 gms. of purified gelatin was added 10 gms. of sodium nitrite along with 15 c.c. of glacial acetic acid. The whole was made up to one litre and

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(1) Hitchcock, J. Gen. Physiology (1923) 95.

left for two days in a thermostat at 25°C. During this time the solution was constantly stirred. The gelatin was then washed repeatedly with distilled water, filtered, and again washed repeatedly with water. Finally it was perfused with absolute alcohol and ether and dried by exposure to the air for four days. In appearance the product was slightly darker in colour than the original purified substance.

Analysis for moisture and ash gave the following results:-

% moisture	(1)	16.91	% ash	(1) 0.06
	(2)	16.85		(2) 0.08
Mean =		16.88%	Mean =	0.07%

REAGENTS.

A.R. or ANALAR silver nitrate, potassium chromate, potassium nitrate, ammonium nitrate, sodium hydroxide and hydrochloric acid were employed throughout the research.

All solutions were prepared with particularly good distilled water.

ADJUSTMENT OF pH.

pH values were determined colorimetrically, the indicators and the buffer solutions varying with the particular range concerned, as shown in the following list

pH range.	Buffer Solutions	Indicator
3.9 - 4.1	Borax, 0.05 N. + Succinic Acid, 0.05 N.	Bromo-cresol green.
4.6 - 5.0	Borax, 0.05 N. + Succinic Acid, 0.05 N.	Bromo-cresol green.
6.9 - 7.1	Sorensen's phosphate Standards.	Phenol red.
8.9 - 9.1	Potassium dihydrogen phosphate, 0.10 N. + Borax, 0.5 N.	B.D.H. indicator "8610" or o-cresol phthalein.
10.0 - 11.0	Borax, 0.05 N. + Sodium Carbonate, 0.05 N.	B.D.H. indicator "9011"

The pH was raised by adding sodium hydroxide and lowered by the addition of hydrochloric acid. The following is the method adopted for adjusting the pH, except in the case of the pH between 10 and 11. Into each of a series of uniform flat-bottomed cylindrical tubes were placed 10 ml. of a 1% gelatin solution and the requisite amount of indicator. Increasing known amounts of standardised sodium hydroxide were added to successive tubes and comparison made with standard buffers lying 0.1 of a pH unit on either side of the

desired value. In this way the proportion of sodium hydroxide or hydrochloric acid to be added to a given amount of gelatin to give a particular pH was estimated. The data given in Table I indicate the amount of sodium hydroxide or hydrochloric acid added to 10 ml. of 1% gelatin.

TABLE I.

Gelatin	Volume (ml.) of 0.02 N. NaOH		
	pH = 6.9 - 7.0	pH = 7.0 - 7.1	pH = 7.0
Gelatin (D)	0.77	0.79	0.78
Hydrolysed	0.53	0.55	0.54
Purified	1.17	1.19	1.18
Deaminated	2.78	2.84	2.81
	pH = 8.8 - 9.0	pH = 9.0 - 9.2	pH = 9.0
Purified (B.D.H. 8610)	1.70	1.83	1.77
(o-cresol phthalein)	1.70	1.80	1.75
	Volume (ml.) of 0.01 N. HCl		
	pH = 4.6 - 4.7	pH = 4.7 - 4.8	pH = 4.7
Purified	0.46	0.50	0.48
	pH = 3.9 - 4.0	pH = 4.0 - 4.1	pH = 4.0
Deaminated	1.24	1.29	1.27

In the case of the purified gelatin, to obtain a pH between 10 and 11, a rough calculation was made of the amount of sodium hydroxide which would be necessary. This

amount was added and the pH then accurately determined by comparison with suitable standards. It was found that 4·50 ml. of 0·178 N. sodium hydroxide were required to bring the pH of 100 ml. of 1·0% gelatin to 10·5. This is equivalent to the addition of 4·01 ml. of 0·02 N. alkali to 10 ml. of the gelatin solution.

To bring the deaminated gelatin to the same pH, 6·0 ml. of 0·178 N. sodium hydroxide were first added to one gramme of gelatin, and the volume made up to 100 ml. with water. This solution was found to have a pH slightly below that required. The extra amount of alkali to be added was ascertained by comparison with standard buffers in the usual way. Actually, the addition of 0·45 ml. of 0·02 N. alkali to 10 ml. of the above 1·0% gelatin solution gave a pH of 10·5. The total amount of sodium hydroxide added was equivalent to the addition of 5·79 ml. of 0·02 N. alkali to 10 ml. of 1·0% gelatin.

The pH values for 1·0% solutions of purified and deaminated gelatin were found to be 4·85 - 4·90 and 4·20 respectively.

PRECIPITATION EXPERIMENTS.

The procedure adopted for ascertaining the amount of gelatin required to inhibit precipitation of silver chromate in mixtures of silver nitrate and potassium chromate was a modification of the method used by Williams and Mackenzie[1] and Bolam and Mackenzie.[2] In each of one series of Pyrex test-tubes were placed:-

y ml. of x N. silver nitrate + (5-y)ml. of water + 5 ml. of z% gelatin,

and in each of another series:-

5 ml. of 0.10 N. potassium chromate + 5 ml. z% gelatin,

the concentration of silver nitrate being varied by altering y. The tubes were placed in a thermostat, the temperature of which was maintained at 25 $\pm$ 0.1°C. by electrical regulation. After half-an-hour, when the solutions had attained the temperature of the thermostat, the contents of each tube in the first series were mixed with the contents of a tube in the second series by transferring the liquid from one tube to the other five or six times. Immediately after mixing, the tubes containing the mixtures were corked, replaced in the thermostat and examined after a period of two hours. To avoid reduction of the silver salts by the action of light, the tubes were wrapped in tin

(1) Williams and Mackenzie, J.C.S., 117 (1920) 844.
(2) Bolam and Mackenzie, Trans. Faraday Soc., 23 (1926) 151.

foil. In all cases where change occurred, it consisted in the appearance of a light-red suspension uniformly distributed throughout the body of the mixture. Given sufficient time, the suspension ultimately settled out.

From the above it will be seen that the composition of any mixture was represented by:-

y ml. x N. $AgNO_3$ + (5-y)ml. H_2O + 5 ml. 0.10 K_2CrO_4 + 10 ml. z% gelatin,

so that the total volume was always 20 ml.

Hence the final concentration of the gelatin = z/2%

" " " " " potassium chromate = 0.025 N.

" " " " " silvef nitrate = $\frac{y \times x}{20}$ equivalents per litre.

In a given series of mixtures there is a value for the concentration of silver nitrate ($\frac{y \times x}{20}$) which just fails to produce precipitation within two hours. This concentration we shall call the "threshold value" of the silver nitrate.

The gelatin was dispersed by heating up to, but never above, 40°C., and any question of deterioration of the gelatin due to bacteriological action was avoided by using freshly prepared solutions. To

ensure reasonable reproducibility of the results, the tubes were thoroughly cleaned before each experiment with hot water, nitric acid and ammonia, and then steamed out and dried in an air oven. Care was taken to avoid scratching the internal surface of the tubes during cleaning operations, since precipitation naturally tends to take place more readily on the scratches than elsewhere.

In general, the presence or absence of precipitation was decided by ordinary visual examination but in some instances confirmation was obtained by means of a Zeiss nephelometer used in conjunction with a Zeiss Pulfrich photometer. This instrument measures the amount of light scattered by a given suspension in terms of a standard source of illumination. The amount of scattered light reaching the eye is controlled by means of a drum-operated shutter, the drum being equipped with a scale. Preliminary adjustment was made with a blank solution where precipitation had definitely not occurred, i.e., a solution containing the appropriate concentrations of gelatin and potassium chromate but no silver nitrate. Only a relatively small amount of light was scattered by this solution. The intensity of the light from the standard source was so adjusted that when the fields of illumination were matched, the scale of the shutter drum gave a reading between 0 and 6. When the blank was replaced by a mixture in which

precipitation had occurred, the amount of scattered light was increased, and on matching the fields by closing the shutter, there was an increase in the scale-reading. Thus, in general, the scale-reading indicated whether precipitation had or had not taken place.

Precautions were taken to keep the mixture at 25° during the nephelometer examination.

A series of precipitation experiments were performed as described above, with stock gelatin at pH of 7.0, with purified gelatin at pH's of 7.0, 9.0 and 10.5, and with deaminated gelatin at pH's of 7.0 and 10.5. The results obtained are given in detail in Table II, which includes the nephelometer readings, and in Tables IV, V, VI.

For reasons to be explained at a later stage, a series of experiments was also carried out with concentrations of potassium chromate greater than 0.025 N. In this case a period of three, instead of two, hours was allowed to elapse before the solutions were examined. The data are shown in Table III.

The significance of the symbols in Tables II, III, IV, V, VI is as follows:

\+ = precipitation

\- = no precipitation

(-) = "Threshold Value" of silver nitrate.

In these tables, as elsewhere, concentrations of reagents are expressed as milliequivalents per litre (m.e./l.) and concentrations of gelatin as grammes dried material per 100 ml. solution (%).

TABLE II.

Precipitation Data for Stock Gelatin

pH = 7.0

Conc. Geln. (%)	Conc. $AgNO_3$ (m.e./l.)	Observation		Nephelometer Readings.
		Expt. 1	Expt. 2	
0.000	0.140	+	+	
	0.135		+	
	0.130	+	+	
	0.125		(-)	
	0.120	(-)	-	
	0.115		-	
	0.110	-		
	0.105			
	0.100	-		
0.001	0.224	+	+	
	0.220	(-)	(-)	
	0.218	-	-	
	0.215	-	-	
	0.213	-		
	0.210	-		
0.00416	0.262	+	+	75.0
	0.256		+	25.0
	0.253		+	16.0
	0.250	(-)	(-)	4.1
	0.247		-	3.4
	0.244			
	0.241			
	0.238	-		

TABLE II. (cont.)

Conc. Geln. (%)	Conc. $AgNO_3$ (m.e./l.)	Observation		Nephelometer Readings.
		Expt. 1	Expt. 2	
0.0416	0.380	+		
	0.370	+		
	0.360	+		
	0.350	+	+	75.0
	0.345		+	58.0
	0.340	(-)	(-)	5.3
	0.335		-	5.3
0.1	0.430	+	+	90·0
	0.425	+	+	30.0
	0.420	+	+	29.0
	0.415	(-)	(-)	10.5
	0.410	-	-	5.3
0.2	0.525	+	+	80.0
	0.519	+	+	64.0
	0.513	+	+	34.0
	0.506	(-)	(-)	4.1
	0.500	-	-	4.0
0.3	0.608		+	58.0
	0.600	+	+	46.0
	0.592	+	+	31.0
	0.583	(-)	(-)	7.3
	0.575	-	-	6.1
0.416	0.708	+		
	0.700	+		
	0.692	+	+	71.5
	0.683	+	+	53.5
	0.676	(-)	(-)	5.7
	0.667	-	-	4.3

TABLE II. (cont.)

Conc.Geln. (%)	Conc. $AgNO_3$ m.e./l.	Observation		Nephelometer Readings
		Expt. 1	Expt. 2	
0.832	1.075	+		
	1.050	+		
	1.025	+	+	
	1.013		+	
	1.000	(-)	+	
	0.988		(-)	
	0.975	-		
1.250	1.383	+	+	71.0
	1.367	+	+	60.0
	1.350	+	+	61.0
	1.333	+	+	54.0
	1.317	(-)	(-)	5.1
	1.300	-	-	4.9
1.664	1.700	+		
	1.675	+		
	1.650	+	+	71.0
	1.625	(-)*	(-)*	50.0
	1.600	-	-	6.0
	1.575	-	-	7.5
	1.550		-	5.0

* Faint signs of precipitation. Considerable precipitation had occurred by the time the mixture was examined in the nephelometer.

TABLE III.

Precipitation Data for Stock Gelatin.
pH = 7.0. Higher Concentrations K_2CrO_4.

Conc. Geln. %	Conc. $AgNO_3$ m.e./l.	Conc. K_2CrO_4 m.e./l.	Observation
1.664	1.000	300	-
		400	-
		500	(-)
		600	+
		700	+
1.664	1.000	700	+
		650	+
		600	+
		500	(-)
1.664	1.000	600	+
		560	+
		530	+
		500	(-)
1.000	0.658	500	-
	0.667		-
	0.675		-
	0.683		(-)
	0.692		+
1.000	0.650	530	-
	0.658		(-)
	0.667		+
	0.675		+
	0.683		+
	0.692		+
	0.700		+

TABLE IV.

Precipitation Data for Hydrolysed Gelatin.

pH = 7.0

Conc. Geln. (%)	Conc. $AgNO_3$ m.e./l.	Observation	
		Expt. 1.	Expt. 2.
0.1 Solution heated on a water-bath for one hour.	0.480	+	
	0.460	+	
	0.440	+	
	0.425		+
	0.420	+	+
	0.415		(-)
	0.410		-
	0.405		-
	0.400	(-)	
0.1 Solution boiled for two hours under reflux	0.480	+	
	0.460	+	
	0.440	+	+
	0.430		(-)
	0.420	(-)	-
	0.410		-
	0.400	-	-
0.832 Solution boiled for two hours under reflux.	1.050	+	
	1.025	+	
	1.000	+	
	0.975	+	+
	0.963		+
	0.950	(-)	+
	0.938		(-)
	0.925		-

TABLE V.

Precipitation Data for Purified Gelatin.

pH	Conc. Geln. (%)	Conc. $AgNO_3$ (m.e./l.)	Observation Expt. 1.	Observation Expt. 2.
7.0	0.416	0.617	+	
		0.608	+	+
		0.600	(-)	(-)
		0.592	-	-
		0.583	-	-
		0.575	-	-
	0.832	1.000	+	
		0.975	+	
		0.950	+	
		0.925	+	
		0.900	+	+
		0.888		+
		0.875	(-)	(-)
		0.863		-
		0.850		-
		0.838		-
	1.250	1.233	+	
		1.200	+	
		1.167	+	+
		1.150		+
		1.133	(-)	(-)
		1.100	-	
		1.067	-	
9.0	1.000	1.250	+	+
		1.200	+	(-)
		1.150	(-)	-
		1.100	-	-
		1.050	-	-

TABLE V. (CONT).

ph	Conc.Geln. (%)	Conc. $AgNO_3$ (m.e./l)	Observation	
			Expt.	Expt. 2.
9	0.500	0.850	+	+
		0.825	+	+
		0.800	+	+
		0.775	(-)	(-)
		0.750	-	-
10.4-10.5	0.500	2.400	+	
		2.350	+	
		2.300	+	+
		2.250	+	+
		2.200	+	+
		2.150	(-)	(-)
	0.750	3.200	+	
		3.133	+	+
		3.100		+
		3.070	(-)	+
		3.037		(-)
		3.000	-	(-)
		2.933	-	
	1.000	4.200	+	
		4.100	+	
		4.050		+
		4.000	+	+
		3.950		(-)
		3.900	(-)	-
		3.850		-
		3.800	-	
		3.700	-	

TABLE VI.

Precipitation Data for Deaminated Gelatin.

ph	Conc. Geln. (%)	Conc. $AgNO_3$ (m.e./l)	Observation Expt. 1	Observation Expt. 2
7	0.500	0.650	+	+
		0.625		(-)
		0.600	(-)	-
		0.550	-	
		0.500	-	
		0.450	-	
		0.400	-	
	1.000	0.950	+	
		0.900	+	+
		0.875		(-)
		0.850	(-)	-
		0.925		-
		0.800	-	-
		0.750	-	
10.5	0.500	2.175	+	+
		2.150	+	+
		2.125	(-)	(-)
		2.100	-	-
		2.075	-	-
	1.000	4.100	+	
		4.000	+	+
		3.950		+
		3.900	(-)	(-)
		3.850		-
		3.800	-	-
		3.700	-	

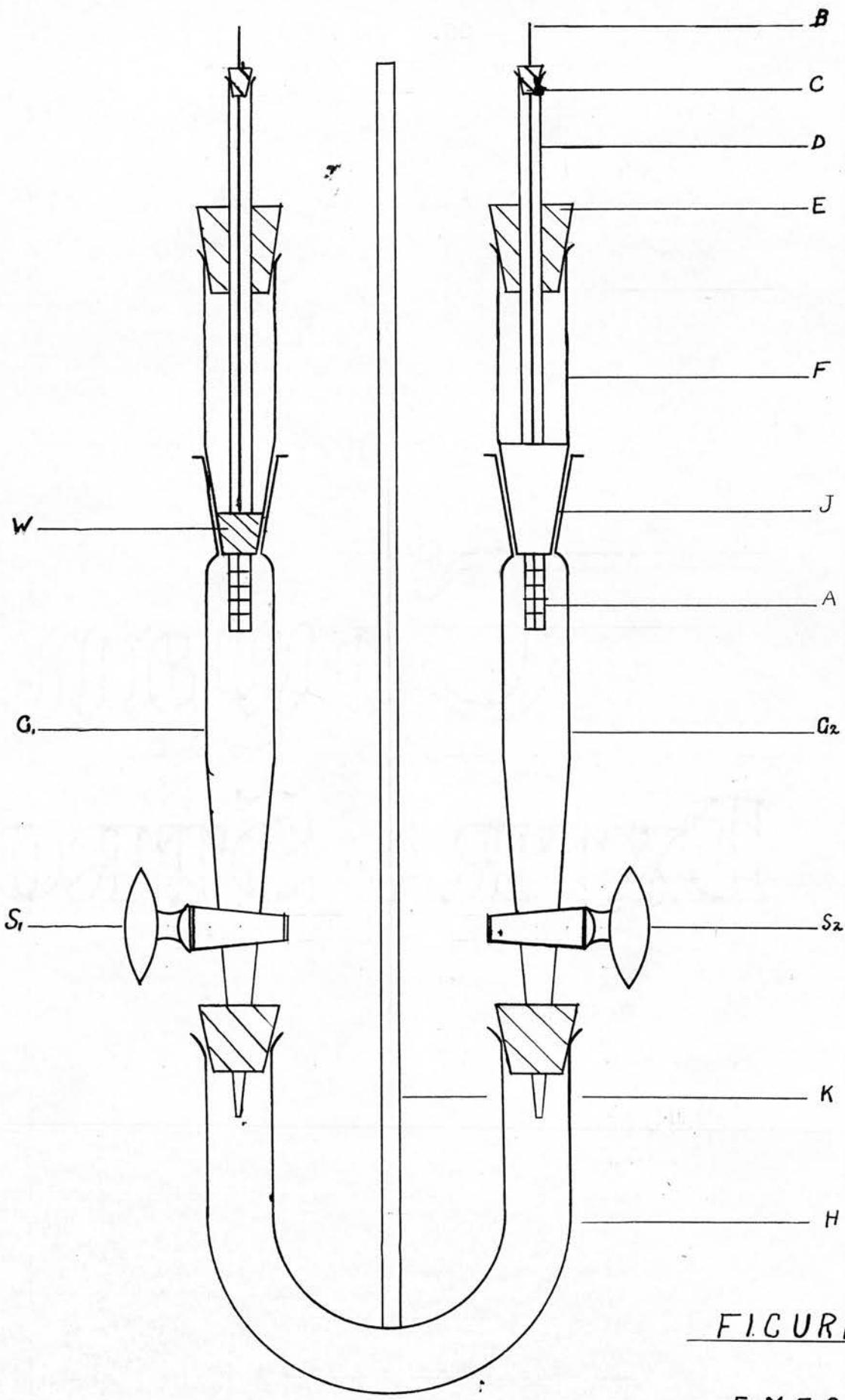


FIGURE I

E.M.F CELL.

E.M.F. MEASUREMENTS.

The activity of the silver ion in various mixtures of gelatin and silver nitrate was obtained by measurement of the e.m.f. of a concentration cell of the type

Ag, 0.1 N. $AgNO_3$, 1OM NH_4NO_3 , x, Ag

where x denotes the system under investigation. Figure I illustrates the construction of the cell used in all the e.m.f. experiments.

Fine, pure silver gauze (supplied by Messrs Johnson, Matthey and Co., London) was used as electrode material. A strip of gauze A was fixed to a piece of stout copper wire B, and held in position in the glass tube D by the rubber stopper C. The whole was held in position in the larger tube F, at one end by means of the rubber stopper E, and at the other by a wad (W) of cotton wool impregnated with paraffin wax which also served to insulate the copper wire from the solution contained in the glass vessel G_1. F and G_1 were connected by a ground joint J.

Connection between the two sides of the cell was effected by means of ammonium nitrate solution contained in the U-tube H. The side tube K allowed for displacement of the ammonium nitrate when G_1 and G_2 were inserted in the limbs of H.

Before use, the electrode was carefully annealed by

heating to dull redness and quenching in cold water, having been previously cleaned by scrubbing with fine sand and dilute nitric acid. New electrodes were prepared for each experiment. It was found from experience that a more steady e.m.f. was obtained if the electrodes were iodised. This was effected by leaving the electrode immersed in an alcoholic solution of iodine for half-an-hour. The electrode was then thoroughly rinsed with alcohol and dried in a stream of air.

The cell was set up as follows. In the first place, H, filled with ammonium nitrate, G_1, filled with 0.1 N. silver nitrate, and G_2, filled with the solution (x) under investigation, were connected together as shown in the diagram. The apparatus was then placed in the thermostat, which was maintained at 25± 0.1°C. throughout the experiments. When it was reasonably certain that all air-bubbles had been expelled from the solutions, the electrodes were placed in position. Since the liquid levels were originally above the necks of G_1 and G_2, these vessels were completely filled with liquid when the electrodes were inserted. Care was also taken to ensure that there were no bubbles of air below the taps S_1 and S_2. These precautions to prevent air-bubbles coming into contact with the electrodes were taken because preliminary experiments showed that they were necessary

in order to obtain a steady e.m.f. The circuit through the cell was completed by opening the taps S_1 and S_2.

The solution (x) contained known concentrations of silver nitrate and gelatin, the gelatin being dispersed as previously described. Only stock gelatin (D) was used in these experiments. Potassium nitrate (0.025 N.) was uniformly present to reduce the resistance of the cell contents, and in every case the pH was adjusted to 7.0. Care was also taken to protect the solutions from any action of light.

The e.m.f. was measured in the usual way, a Tinsley ionisation potentiometer being used in conjunction with a sensitive Broca galvanometer. The Pye standard cadmium cell was checked at intervals against a second standard certified by the National Physical Laboratory as giving an e.m.f. of 1.01835 volts at 15°C. The first reading was taken as quickly as possible after the electrodes were immersed, and subsequent readings at half-hour intervals. Table VII gives in detail the data obtained.

TABLE VII.

E.M.F. Data.

Conc.Geln. (%)	Conc.$AgNO_3$ (m.e./l.)	E.M.F. (millivolts)			
		Expt. 1		Expt. 2.	
		Half-hour Readings.	Mean	Half-hour Readings	Mean.
0.25	1.000	120.9 119.8 119.4 120.0 121.5 117.4	119.8	121.0 119.4 119.4 119.2	119.6
0.624	1.000	130.2 131.2 131.0 131.0 130.0 129.4 130.2 130.2	130.4	129.9 130.2 130.6 130.3	130.3
1.024	1.000	142.2 141.2 140.8 140.8 141.2 141.7 141.8 141.0	141.3	138.8 139.0 140.3 140.6 139.8	139.9
1.374	1.000	158.8 159.4 159.6 159.8 160.3 160.0 159.7	159.7	158.8 159.2 158.9	159.0

TABLE VII. (cont.)

Conc.Geln. (%)	Conc. $AgNO_3$ (m.e./l)	E.M.F. (millivolts) Expt. 1 Half-hour Readings.	Expt. 1 Mean	Expt. 2. Half-hour Readings.	Expt. 2. Mean.
0.2*	0.506	138.8 139.6 139.8 139.6 139.5	139.6	139.4 139.3 139.3	139.3
0.3*	0.583	138.3 138.2 138.2 138.2	138.2	138.8 138.8 138.7 138.8	138.8
0.416*	0.675	138.0 138.0 138.0 138.0 138.0	138.0	139.5 138.2 138.3 138.2	138.4
0.832*	1.000	135.4 135.4 135.4 135.6 135.6	135.5	136.4 136.2 136.2 136.2 136.2	136.2
1.250*	1.317	137.4 138.4 138.2 138.2 138.4 138.4	138.3	138.9 137.1 137.2 137.1	137,1
1.664*	1.625	137.4 137.4 137.5 137.5 137.4	137.4	137.8 137.2 137.1 137.2	137.3

* Conditions same as for the precipitation experiments, except that potassium chromate was replaced by potassium nitrate.

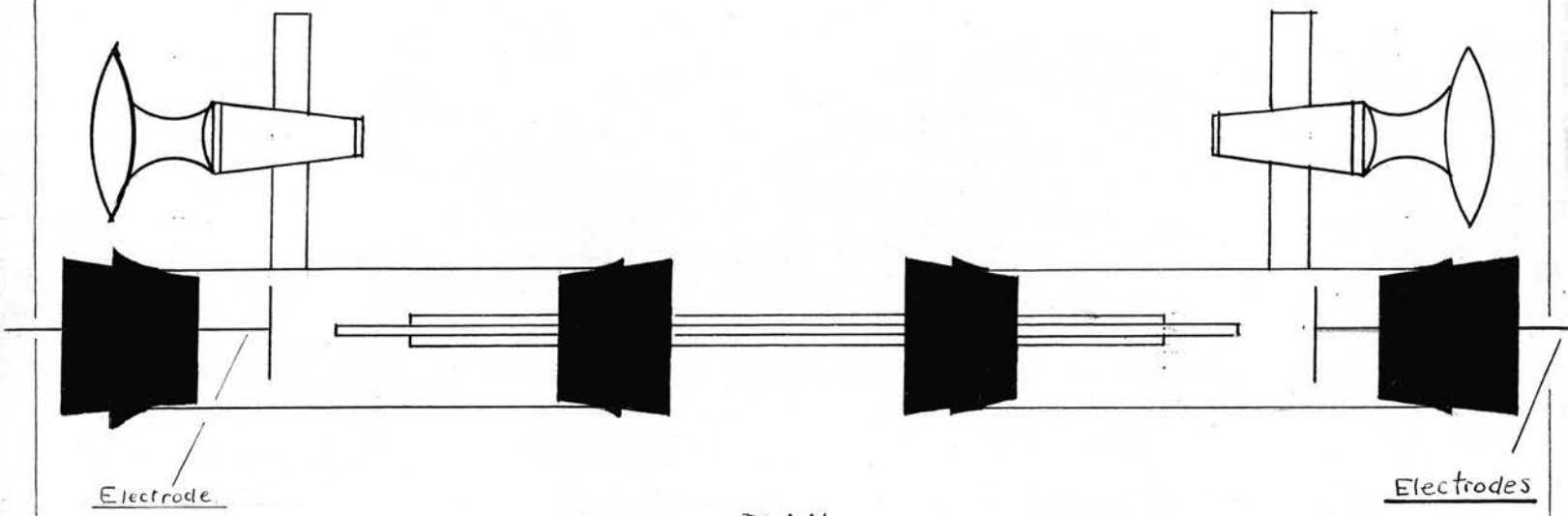
TABLE VII. (cont.)

Conc.Geln. (%)	Conc.$AgNO_3$ (m.e./l.)	E.M.F. (millivolts) Expt. 1. Half-hour Readings.	Mean	Expt. 2, Half-hour Readings.	Mean
0.832	0.506	168.8 168.8 168.7 168.8	168.8	169.6 169.6 169.5 169.5	169.6
0.832	0.583	159.2 159.2 159.3 159.2	159.2	159.8 159.8 159.8	159.8
0.832	0.675	155.6 155.4 155.4 155.4 155.4	155.4	155.0 155.0 155.0	155.0
0.832	1.320	125.8 126.0 125.9 126.2	126.0	126.4 126.5 126.5 126.6 126.5	126.5
0.416	1.320	117.2 116.9 116.5 117.2 117.2 117.3 116.8	117.0	116.6 116.5 116.5 116.5	116.5
1.000	1.630	123.6 123.5 123.1 123.5	123.4	122.8 122.8 122.7	122.8

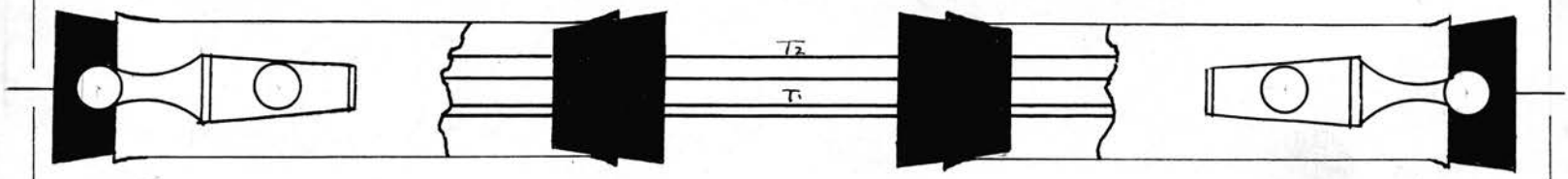
TABLE VII. (cont.)

Conc.Geln. (%)	Conc.$AgNO_3$ (m.e./l.)	E.M.F. (millivolts) Expt. 1. Half-hour Readings.	Expt. 1. Mean	Expt. 2. Half-hour Readings.	Expt. 2. Mean
0.416	1.630	110.2 109.6 109.6 109.6	109.6	108.8 108.8 108.8	108.8
0.822	14.25	52.0 50.2 50.0 51.0	50.8	50.4 50.9 51.1 51.1	50.9

SIDE VIEW



PLAN



OPTICAL ARRANGEMENT

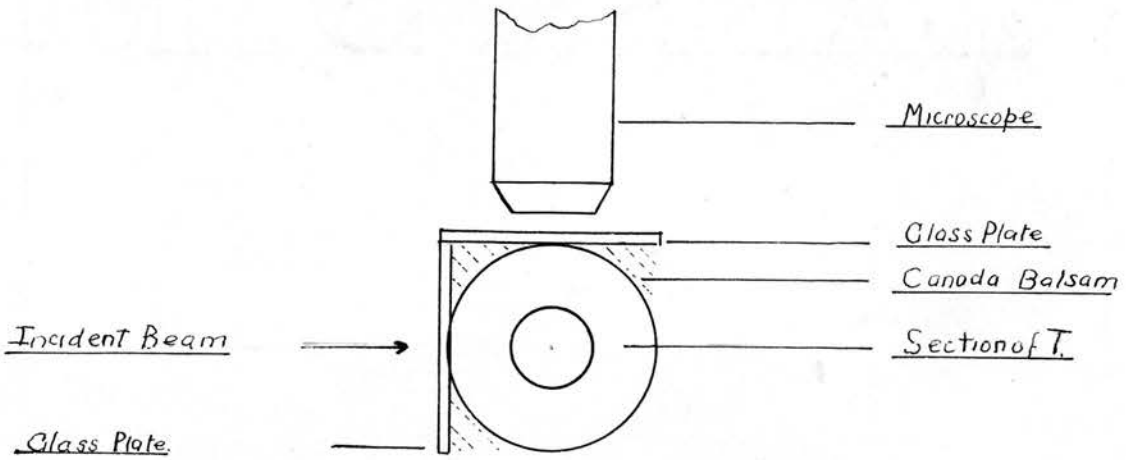


FIGURE II

ELECTROPHORESIS CELL

ELECTROPHORESIS DATA.

Measurement of Electrophoretic Mobility.

Apparatus. Measurements were made of the electrophoretic mobility of particles of silica suspended in solutions of gelatin, with and without the addition of silver nitrate.

An unusual type of cell, devised by Smith and Lisse,[1] was employed. The essential parts of the apparatus are shown in Figure II. T_1 and T_2 were parallel quartz capillary tubes of specific dimensions. They were held by rubber stoppers which fitted into wide tubes of Pyrex glass. The end tubes were provided with Pyrex stopcocks for filling and with electrodes consisting of silver gauze fused to silver wires, which passed through the rubber stoppers.

The dimensions of the quartz tube were such that

$$L_2/L_1 = A^2 (A^2 - 2)$$

where $A = R_2/R_1$, and L_2, L_1, R_2 and R_1, are the lengths and radii of tubes T_2 and T_1 respectively, T_1 having a smaller radius than T_2. Smith and Lisse have shown that under these conditions there is no movement of liquid along the axis of the tube with the smaller radius, due to electroendosmosis. Hence the observed velocities of particles at this depth are the actual electrophoretic velocities of the particles. Since

(1) Smith and Lisse, J. Physical Chem., 40 (1936) 399. See, also, Abramson, Trans. Faraday Soc., 36 (1940)

the velocity gradient is small near the correct level of observation, slight errors in fixing the level are less important in the two-tube than in the usual one-tube cell. Moreover, focussing is easier, since the observed velocity is a maximum at the correct level. Thus the Smith and Lisse cell has certain advantages over the older type.

Smith and Lisse determined values for the electrophoretic mobilities of quartz particles in distilled water by means of both the double-tube cell and the tube T_1 used as a single-tube cell, the observations being made in each case at the depth where theoretically there should be no movement of the liquid. There was no significant difference between the values obtained.

The dimensions of the tubes used in the present work were as follows:-

L_1 = 12.2 cm.

L_2 = 10.04 cm.

R_1 = 0.622 mm.

R_2 = 0.955 mm.

In the cell actually used by Smith and Lisse the tube T_1 was ground near the centre to give plane faces for proper illumination. This procedure was tried at first in the present series of experiments, but the tubes broke so easily during the assembly of the cell that attempts were made to find an alternative method. It was found that satisfactory results could be obtained

by cementing thin plates of glass on to the tube by means of Canada balsam, as shown in Figure II. Dark background illumination was used, and the movement of the particles was observed by means of a high-power Zeiss microscope provided with a micrometer scale in the eyepiece.

Procedure. Pure precipitated silica was further reduced by grinding in an agate mortar. The powder was shaken up with distilled water, and the suspension allowed to stand for one hour to eliminate the coarser particles. The supernatant liquid was then removed by decantation and used to prepare the experimental mixtures by the addition of gelatin and the other substances involved. In all cases the suspension was made 0.5% with respect to gelatin. Sodium nitrate was added in most instances to adjust the ionic strength.

A difference of potential of 220 volts, measured by a voltmeter in parallel with the cell, was maintained between the electrodes. As Smith and Lisse have pointed out, the fall of potential through the wide end-tubes can be neglected so that the potential gradient through T_1 in the present work was $220/L_1 = 220/12.2$ volts. The current was reversed after every 5th reading. Between experiments, the cell was taken apart and thoroughly cleaned with chromic mixture and warm distilled water.

The experimental data are given in detail in

Tables VIII(a) to VIII(e), where the figures represent the time in seconds taken by a particle to travel $0.01 \times 10/11$ cm.

Purified gelatin only was used in this experiment.

TABLE VIII. (a)

pH = 7.0

$AgNO_3$ = 2.15 m.e./l. NaOH = 1.18 " " Total Electrolyte = 3.33 " "	NaOH = 1.18 m.e./l. Total Electrolyte = 1.18 " "
Expt.	Expt.
8.2	7.0
8.8	7.6
8.2	7.4
8.0	7.2
8.2	7.5
8.0	7.1
7.9	7.0
8.1	7.6
8.2	7.1
7.9	7.3
8.3	6.9
8.4	7.0
8.6	7.6
7.9	7.3
7.8	7.2
8.1	7.2
8.2	7.4
8.5	7.5
7.9	7.1
8.2	7.0
Mean = 8.2	Mean = 7.2

Motion was towards the positive pole.

TABLE VIII. (b)

pH = 7.0

$NaOH$ = 1.18 m.e./l. $AgNO_3$ = 0.74 " " $NaNO_3$ = 4.50 " " Total Electrolyte) = 6.42 " "		$NaOH$ = 1.18 m.e./l. $NaNO_3$ = 5.25 " " Total Electrolyte) = 6.43 " "	
Expt. 1.	Expt. 2.	Expt .1.	Expt. 2.
9.2	9.8	9.0	9.2
9.5	9.2	8.5	9.0
10.8	9.4	9.6	8.7
9.5	9.6	9.4	8.8
9.3	10.0	8.2	8.7
10.0	9.0	9.6	9.0
9.4	9.7	9.7	9.5
9.4	9.8	8.5	9.1
9.2	9.2	8.6	8.6
9.7	9.3	9.0	8.5
9.5	9.8	9.1	8.5
9.3	9.4	8.2	9.0
9.9	9.3	8.4	9.1
9.9	9.5	9.2	9.2
9.7	9.5	8.5	8.4
9.2	9.6	8.4	8.7
9.8	9.2	8.7	9.0
9.5	9.7	9.1	8.4
9.3	9.6	8.5	8.4
9.6	9.2	9.0	8.5
Mean 9.5	Mean 9.5	Mean 8.9	Mean 8.7

Motion was towards the positive pole.

TABLE VIII. (c)

pH = 7.0

NaOH = 1.18 m.e./l. $AgNO_3$ = 14.32 " " Total Electrolyte = 15.50 " "		NaOH = 1.18 m.e./l. $NaNO_3$ = 14.32 m.e./l. Total Electrolyte = 15.50 " "	
Expt. 1.	Expt. 2.	Expt. 1.	Expt. 2.
10.4	10.6	9.6	9.4
10.6	10.9	9.4	9.5
10.6	10.0	9.3	9.5
10.7	10.2	9.6	9.6
10.3	10.0	9.7	10.0
10.2	9.9	10.0	10.0
10.6	10.4	10.0	9.2
10.3	10.5	9.3	9.2
10.0	10.5	9.4	9.1
9.9	10.6	9.1	10.0
10.5	10.7	9.5	9.9
10.8	10.7	9.4	9.4
10.9	10.0	9.4	9.5
10.2	9.7	9.3	9.3
10.3	10.7	9.6	9.2
10.5	10.6	9.0	9.2
10.6	10.0	9.3	10.0
10.6	10.7	9.3	9.6
10.3	10.0	9.9	9.5
10.3	9.9	10.0	9.3
	10.7		9.3
Mean 10.4	Mean 10.3	Mean 9.6	Mean 9.5

Motion was towards the positive pole.

TABLE VIII. (d)

pH = 10.4 - 10.5

Na OH = 4.27 m.e./l. $AgNO_3$ = 2.15 " " Total Electrolyte) = 6.42 " "		NaOH = 4.27 m.e./l. Total Electrolyte) = 4.27 " "	
Expt. 1.	Expt. 2.	Expt. 1.	Expt. 2.
7.0	5.8	5.6	6.1
6.8	6.4	6.0	5.4
7.8	6.4	6.5	5.3
6.0	7.6	6.0	6.2
6.8	5.6	6.2	5.6
6.8	6.2	5.3	6.7
6.0	6.4	5.9	5.5
5.8	6.4	6.5	5.6
6.8	6.1	5.5	6.2
5.8	6.4	5.5	5.9
5.5	6.1	5.8	5.5
7.5	6.5	5.8	5.4
5.9	6.0	6.5	5.4
5.5	5.9	5.5	5.5
5.0	6.2	5.6	5.5
5.5	6.3	5.8	5.7
6.0	6.4	5.3	5.8
6.5	6.4	5.6	6.1
6.2	6.5	5.5	5.5
5.5	6.4	6.2	5.5
Mean 6.2	Mean 6.3	Mean 5.8	Mean 5.7

Motion was towards the positive pole.

TABLE VIII. (e)

pH = ca. 4.0

HCl = 4.00 m.e./l.	
Expt. 1.	Expt. 2.
10.6	9.7
9.7	10.3
10.2	9.6
9.6	9.5
10.0	10.2
9.5	9.2
10.0	9.3
10.3	10.4
9.4	9.7
9.5	9.5
10.2	10.1
9.6	10.1
9.2	9.5
9.7	9.6
9.8	10.0
10.0	9.6
9.6	10.0
9.6	10.5
10.0	9.8
10.0	9.6
Mean 9.8	Mean 9.8

Motion was towards the negative pole.

ELECTRO-ENDOSMOSIS.

After consideration of the results obtained from the electrophoretic data it was decided that measurdments of the electroendosmotic flow through gelatin gels might possibly yield valuable information. Accordingly, the apparatus shown in Figure III was set up. It consisted of a U-tube fitted with side arms which were connected to each other through a capillary tube (A), about 8 cm. in length. Two graduation lines (G) were marked on the U-tube. The electrodes (E) consisted of platinum wire wound spirally and sealed into the glass tubes (T), which were held in position by means of the rubber stoppers (S). A potential difference was imposed on the electrodes through the copper wires (C) which dipped into mercury (H) in the bottom of the tubes, thus completing the connection with the electrodes. An aperture (D) was blown in the glass tube T to enable any gas evolved by electrolysis to escape. Soda lime (L), loosely packed in the tube T, insulated the U-tube from the carbon dioxide of the atmosphere.

For each complete experiment two solutions were necessary, namely, a gelatin solution with or without silver nitrate, and the corresponding external solution. The concentrations of the electrolytes in the solution

outside the gel were adjusted so that the solution was sufficiently near to equilibrium with the gel for the present purpose.

The apparatus was operated as follows. The gelatin solution, the gelatin concentration of which was always 2%, was run into the U-tube up to the graduation marks, and cooled by placing the tube in a bath of ice-water. When the solution had gelled, the upper portions of the U-tube and the capillary (A) were filled with the external solution. A bubble of air was then introduced into the capillary tube by means of a bent glass tube. The electrodes were now inserted, being brought as near as possible to the surface of the gel in order to avoid the necessity of taking into account the resistance of the external solution.

When the current was switched on, a difference in hydrostatic pressure was set up between the two arms of the U tube as the result of the electroendosmotic flow of liquid through the gel. The difference in hydrostatic pressure caused the bubble to move alongside the capillary C in a direction which indicated that the water was positively charged.. When the current was reversed, the flow took place in the opposite direction.

The rate at which the bubble moved was obviously a measure of the rate of flow of the liquid through

the gel. The figures given under "time of flow" in the following tables (IX (a) - (e)) are the times (in seconds) taken by the bubble to travel a distance of 3 cm., with a difference of potential of 100 volts between the electrodes, which were 10·5 cm. apart. The current was reversed after each reading.

The U-tube was kept immersed in ice-water throughout each experiment.

It will be realised that in order to obtain a ready flow of liquid through the capillary tube, the whole apparatus must be scrupulously clean. This was ensured by treatment with chromic acid and distilled water.

It will be seen from Tables IX (a) - (e) that the total concentration of electrolyte added to the gel was uniformly 23·66 m.e./l. in the case of purified gelatin, and 29·5 in that of the deaminated material. These figures will also represent the values of the ionic strength if the gelatin combines with hydroxyl and silver ions to form charged groups whose contribution to the ionic strength is the same as that of an equal number of simple monovalent ions. If this is the case, the ionic strength may be regarded as constant, since the difference between 23·66 and 29·5 would be insignificant in the present connection.

In every case the composition of the external solution was so chosen that the pH and the concentration

of sodium nitrate were the same as in the gel, and the concentration of silver nitrate was equal to that of the free silver in the gel. Thus the external solution was not in true equilibrium with the gel, since no allowance was made for the Donnan effect. However, the results indicated that the systems were near enough to equilibrium for the purpose in view.

In Table XV (page 87) will be found values for the "rate of flow", derived from the time of flow according to the formula:

$$\text{Rate of flow} = \frac{3 \times 10{\cdot}5 \times 10^4}{\text{mean time of flow} \times 100} \mu/\text{sec./volt/cm.}$$

ELECTRO-ENDOSMOSIS DATA.

TABLE IX(a).

Purified Gelatin at pH = 7.0.

Without $AgNO_3$		With $AgNO_3$	
External Wolution	Gel	External Solution	Gel
$NaNO_3$ = 19.03 m.e./l. *T.E. = 19.03 m.e./l.	NaOH = 4.63 m.e./l. $NaNO_3$ = 19.03 " " T.E. = 23.66 " "	$NaNO_3$ = 17.45 m.e./l. $AgNO_3$ = 0.36 " " T.E. = 17.81 " "	NaOH = 4.63 m.e./l. $AgNO_3$ = 1.58 " " $NaNO_3$ = 17.45 " " T.E. = 23.66 " "
Time of Flow (seconds)			
Expt. 1	Expt. 2	Expt. 1.	Expt. 2
354 364 366 356	348 358 362 356 366 360	512 522 520 518	520 516 524 525 517 516
Mean 360	358	518	520

* T.E. = Total electrolyte.

TABLE IX(b).

Purified Gelatin at pH = 9.0

Without $AgNO_3$		With $AgNO_3$	
External Solution	Gel	External Solution	Gel
NaOH = 0.01 m.e./l. $NaNO_3$ = 16.62 " " T.E. = 16.63 " "	NaOH = 7.04 m.e./l. $NaNO_3$ = 16.62 " " T.E. = 23.66 " "	NaOH = 0.01 m.e./l. $AgNO_3$ = 0.36 " " $NaNO_3$ = 14.60 " " T.E. = 14.97 " "	NaOH = 7.04 m.e./l. $AgNO_3$ = 2.01 " " $NaNO_3$ = 14.60 " " T.E. = 23.65 " "
Time of Flow (seconds).			
Expt. 1	Expt. 2	Expt. 1	Expt. 2
337 343 357 356 350 350	348 352 349 354	563 562 559 555 564 559	564 570 566 559 559 564
Mean = 348	351	560	564

TABLE IX(c).

Purified Gelatin at pH = 10.5.

Without $AgNO_3$		With $AgNO_3$	
External Solution	Gel	External Solution	Gel
NaOH = 0.32 m.e./l. $NaNO_3$ = 7.64 " " T.E. = 7.96 " "	NaOH = 16.02 m.e./l. $NaNO_3$ = 7.64 " " T.E. = 23.66 " "	NaOH = 0.32 m.e./l. $AgNO_3$ = 0.36 " " T.E. = 0.68 " "	NaOH = 16.02 m.e./l. $AgNO_3$ = 7.64 " " T.E. = 23.66 " "
Time of Flow (seconds).			
Expt. 1	Expt. 2	Expt. 1	Expt. 2
123 115 114 120 123 125	114 120 115 121 116 123	240 232 238 230 220 230	226 230 232 227 231 239
Mean = 120	118	232	231

TABLE IX(d).

Deaminated Gelatin at pH = 7.0.

Without $AgNO_3$		With $AgNO_3$	
External Solution	Gel	External Solution	Gel
$NaNO_3$ = 18.24 m.e./l. T.E. = 18.24 " "	$NaNO_3$ = 18.24 m.e./l. NaOH = 11.25 m.e./l. T.E. = 29.49 " "	$AgNO_3$ = 0.36 M.e./l. $NaNO_3$ = 16.90 " " T.E. = 17.26 " "	NaOH = 11.25 m.e./l. $NaNO_3$ = 16.90 " " $AgNO_3$ = 1.64 " " T.E. = 29.49 " "
Time of Flow (seconds)			
Expt. 1	Expt. 2	Expt. 1	Expt. 2
150 160 150 152	152 159 153 150	150 160 156 156	155 148 160 158
Mean = 153	154	156	153

1·34

TABLE IX(e).

Deaminated Gelatin at pH = 10.5

Without $AgNO_3$		With $AgNO_3$	
External Solution	Gel	External Solution	Gel
NaOH = 0.32 m.e./l. $NaNO_3$ = 7.44 " " T.E. = 7.76 " "	NaOH = 22.10 m.e./l. $NaNO_3$ = 7.44 " " T.E. = 29.54 " "	NaOH = 0.32 m.e./l. $AgNO_3$ = 0.36 " " T.E. = 0.68 " "	NaOH = 22.10 m.e./l. $AgNO_3$ = 7.44 " " T.E. = 29.54 " "
Expt. 1	Expt. 2	Expt. 1	Expt. 2
83 85 80 81	87 89 80 86	83 80 86 87	87 89 85 83
Mean = 82	85.3	84	86

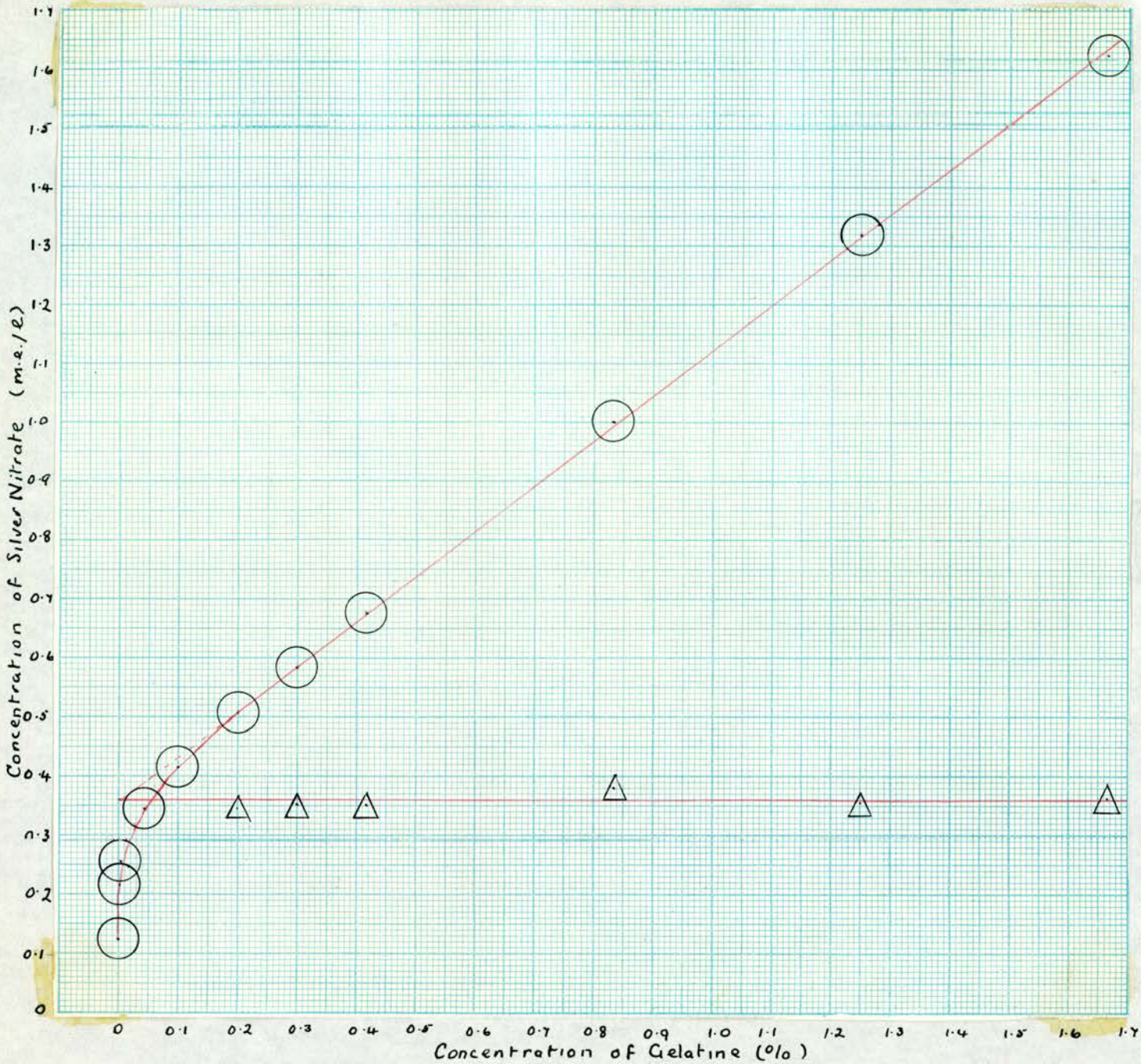


FIGURE IV

PRECIPITATION CURVE - STOCK GELATIN AT pH 7.0

○ Threshold Concentration of Silver Nitrate

△ Concentration of Free Silver as determined by E.M.F. experiments

D I S C U S S I O N.

The Precipitation Curve.

It is necessary to consider, in the first place, the influence of the stock gelatin upon the precipitation of silver chromate, the relevant data for which are given in Table II (p. 18).* In these experiments the threshold concentrations of the silver nitrate were determined by both direct observation and nephelometric examination. A survey of the data shows that the two methods agreed closely, and that by the direct method the threshold value in any given case could be readily determined to within about 1 per cent., which was sufficient for the present purpose. Direct observation only was therefore employed for the other series of precipitation experiments, since it is the more rapid and convenient method.

The most probable valuesfor the threshold concentrations, as derived from Table II, are collected in Table X (p. 56), and plotted in Figure IV. Thus the upper curve in the figure shows the manner in which the concentration of silver nitrate, which just fails to produce precipitation with 25.0 m.e./l. potassium

*The data obtained in preliminary precipitation experiments carried out for purposes of orientation are not included in Tables II to VI.

TABLE X.

Threshold Values of Silver Nitrate.

Gelatin	pH	Conc. K_2CrO_4 (m.e./l.)	Conc. gelatin (%)	Threshold value silver nitrate (m.e./l.)
Stock	7.0	25.0	0.000	0.127
	"	"	0.001	0.222
	"	"	0.00416	0.252
	"	"	0.0416	0.343
	"	"	0.100	0.415
	"	"	0.200	0.506
	"	"	0.300	0.583
	"	"	0.416	0.675
	"	"	0.832	1.002
	"	"	1.250	1.317
	"	"	1.664	1.625
Hydrolysed	7.0	25.0	0.100 (water bath)	0.415
	"	"	0.100 (boiled)	0.435
	"	"	0.832 (boiled)	0.950
Stock	7.0	515.0	1.000	0.675
	"	"	1.664	1.000

chromate in two hours, varied with the concentration of stock gelatin, when at pH = 7.0. It will be seen that this curve, which may be called for convenience the "precipitation curve", continuously bends away from the y-axis as the gelatin concentration increases to about 0.2 per cent., and thereafter follows a linear course with further increase in gelatin. Extension (dotted line in figure) of the linear portion of the curve to the y-axis gives 0.36 m.e./l. as the value of the intercept. The following appear to be reasonable deductions from the shape of the curve, which is typical of all the precipitation curves encountered in the present work.

(I) Part of the silver is combined with the gelatin and the remainder exists as free (simple) ions in the solution.

(II) The concentration of free silver increases with increase in the gelatin concentration to about 0.2 per cent., and thereafter remains constant at 0.36 m.e./l. with further increase in the gelatin.

(III) At a given value of the free silver (in this case 0.36 m.e./l.) the amount of silver combined with unit weight of gelatin is the same, whatever the concentration of the gelatin.

(IV) Under the given conditions the concentration of free chromate ion is the same in all cases, i.e., any variation due to combination with the gelatin is of

negligible proportions. According to the foregoing, the interpretation of the linear portion of the precipitation curve may be expressed concisely in the following manner.

$$\text{Milli-equivalents of silver combined with one gm. of gelatin}$$

$$= \frac{\text{Threshold value - concentration free silver}}{\text{Concentration of gelatin}}$$

$$= \frac{\text{Total conc. silver nitrate - 0.36}}{\text{Concentration of gelatin}} = \text{constant}$$

In Figure IV are also shown the concentrations of free silver calculated from the e.m.f. values obtained with mixtures of silver nitrate and stock gelatin at the same concentrations as for the precipitation experiments covering the linear portion of the precipitation curve, and with 25 m.e./l. of potassium nitrate present instead of potassium chromate. The actual readings are given in Table VII (p. 31), from which it will be seen that the e.m.f. was very steady, the variation being in most cases less than one milli-volt during a period of from two to three hours.* In Table XI are collected the mean e.m.f. values obtained from duplicate experiments, together

*This applies to all experiments in Table VII. In some instances the cell was left overnight and the value of the e.m.f. found to be unaltered. In a few experiments the e.m.f. decreased very rapidly from the start. The results of these experiments are not included in Table VII, since they are obviously unreliable.

with derived values of the activity (a_{Ag^+}) and concentration of the free silver ion. The activity was calculated from the e.m.f. by means of the formula:-

$$\text{e.m.f.} = 0.0591 \log 0.077 / a_{Ag^+}$$

where 0.077 = the activity of silver ion in 0.1 N. silver nitrate, according to Lewis and Randall.(1) The concentration was derived from the activity by the formula:-

$$\text{Concentration } Ag^+ = a_{Ag^+} / f_{Ag^+}$$

where f_{Ag^+} = the value of the activity coeffieient in a pure solution of silver nitrate of silver ion activity a_{Ag^+}. For the systems under consideration, f_{Ag^+} was taken as unity.

The concentrations of free silver obtained in the above manner are plotted in Figure IV and will be found to lie within 7 per cent. of a line drawn parallel to the x-axis and at the level of the intercept (0.36 m.e./l.) on the y-axis of the linear portion of the precipitation curve. It appears reasonable to draw the following main conclusions from this result.

(1) The interpretation given above of the shape of the precipitation curve is confirmed by the potentiometric data.

(2) The proportions of combined and free silver in mixtures of gelatin and silver nitrate may be determined

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(1) Lewis and Randall, "Thermodynamics" (1923) p. 382.

by the silver electrode at pH = 7.0, in the manner described.

(3) The precipitation technique offers an independent method of determining the proportions of combined and free silver.

Further evidence in support of these conclusions will emerge during the course of the discussion.

In calculating the concentration of free silver ion from the activity, it was assumed that the influence of the gelatin up to a concentration of 1.664 per cent., and of the potassium nitrate (25.0 m.e./l.), upon the activity coefficient was negligible. This assumption needs justification, since the potassium nitrate alone might be expected to reduce the activity coefficient to about 0.85 according to Lewis and Randall's figures. Apart from the justification afforded by the agreement of the values for the concentration of free silver, as calculated from the e.m.f. data and as deduced from the precipitation curve in the manner described, evidence was provided by e.m.f. determinations carried out with solutions containing only silver nitrate and potassium nitrate. The following results were obtained.

Concentration of silver nitrate (m.e./l.)	Concentration of potassium nitrate (m.e./l.)	E.M.F.	
		Observed	Calculated.
0.494	27.2	129.4 129.5	130.6
0.286	11.06	141·6 141·1	143.3

The calculated values for the e.m.f. were derived on the assumption that the solutions contained the silver nitrate alone. It will be seen that the experimental values tend to be lower than the calculated, instead of two to four milli-volts higher, as would be the case if the e.m.f. was calculated on the assumption that the activity of the silver ion depended upon the total ionic strength of the solution, and not simply on the concentration of the silver nitrate. This anomaly has been observed by Bolam and Mackenzie,(1) and apparently also by others, since Carrolland Hubbard(2) remark that "The whole calculation of the concentration of silver ion from its activity in the presence of other electrolytes is open to some question."

Since the concentration of ionised gelatin groups in the more concentrated solutions is quite considerable,* the absence of any measurable influence of the gelatin upon the activity of the silver ion supports the view that the contribution of a (polyvalent) protein ion to the ionic strength is not greater than that of an equivalent number of univalent ions. This is usually assumed, on the ground that the charged groups of the protein ion are separated by distances considerably greater than the reciprocal length of the Debye-Hückel

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(1) Bolam and Mackenzie, Trans. Faraday Soc., 22 (1926) 165.
(2) Carrolland Hubbard, U.S. Bureau of Standards, of Research, 7 (1931) 820.
* See later (p. 92).

TABLE XII.

Stock gelatin at pH = 7·0.

E.M.F. Data.

Conc. $AgNO_3$ (m.e./l.)	Conc. gelatin (%)	E.M.F. (m.v.)	$a_{Ag^+} \times 10^3$	f_{Ag^+}	Conc. free Ag^+ (m.e./l.)	Conc. combined Ag (m.e./l.)	Milli-equiv.Ag combined with 1 gm. gelatin	C**
0.506	0.832	169.2	0.106	1.000	0.106	0.400	0.0481	3.155
"	0.200	*139.5	0.332	"	0.332	0.174	0.0870	1.486
0.583	0.832	159.5	0.154	"	0.154	0.429	0.0516	2.852
"	0.300	*138.5	0.349	"	0.349	0.234	0.0780	1.636
0.675	0.832	155.2	0.182	"	0.182	0.493	0.0593	2.435
"	0.416	*138.2	0.354	"	0.354	0.321	0.0772	1.647
1.000	1.374	153.0	0.199	"	0.199	0.801	0.0583	2.447
"	1.024	140.6	0.322	"	0.322	0.678	0.0662	1.967
"	0.832	*135.9	0.386	"	0.386	0.614	0.0738	1.678
"	0.624	130.4	0.479	0.980	0.489	0.511	0.0819	1.387
"	0.250	119.7	0.726	0.974	0.745	0.255	0.1018	0.864
1.317	1.250	*137.7	0.360	1.000	0.360	0.957	0.0766	1.652
"	0.832	126.3	0.562	0.978	0.575	0.742	0.0892	1.177
"	0.416	116.8	0.813	0.973	0.836	0.481	0.1155	0.683
1.625	1.664	*137.4	0.364	1.000	0.364	1.261	0.0758	1.664
"	1.000	123.1	0.637	0.976	0.653	0.972	0.0972	1.000
"	0.416	109.2	1.094	0.970	1.128	0.497	0.1195	0.416
5.000	1.248	86.3	2.670	0.952	2.804	2.196	0.1760	
14.230	0.832	50.9	10.690	0.900	11.880	2.350	0.2822	

* Conditions same as in precipitation experiments, except that potassium chromate was replaced by potassium nitrate.

theory.[1]

The concentration of potassium chromate was given the relatively high value of 25.0 m.e./l. and the pH was brought to 7.0 in the hope that any combination of the chromate ion with the gelatin would be so small that variation in the amount of combination with change in the gelatin concentration would not alter the concentration of free chromate ion to any appreciable extent. The choice of conditions was suggested by the work of Bolam and Donaldson,[2] who showed that the combination of chromate ion with gelatin decreased with increase in the pH. Moreover, the data of these workers gave some idea of the concentration of potassium chromate which would be necessary to attain the end in view. It is concluded that the concentration of free chromate ion actually was constant in the precipitation experiments, for it is difficult to see how otherwise the interpretation of the quantitative behaviour of the gelatin and silver nitrate given above could be valid.

The E.M.F. Data.

It will be convenient to consider at this point the e.m.f. measurements as a whole. The data are given in detail in Table VII (p. 30), and the final results are shown in Table XII (p. 63). Concentrations

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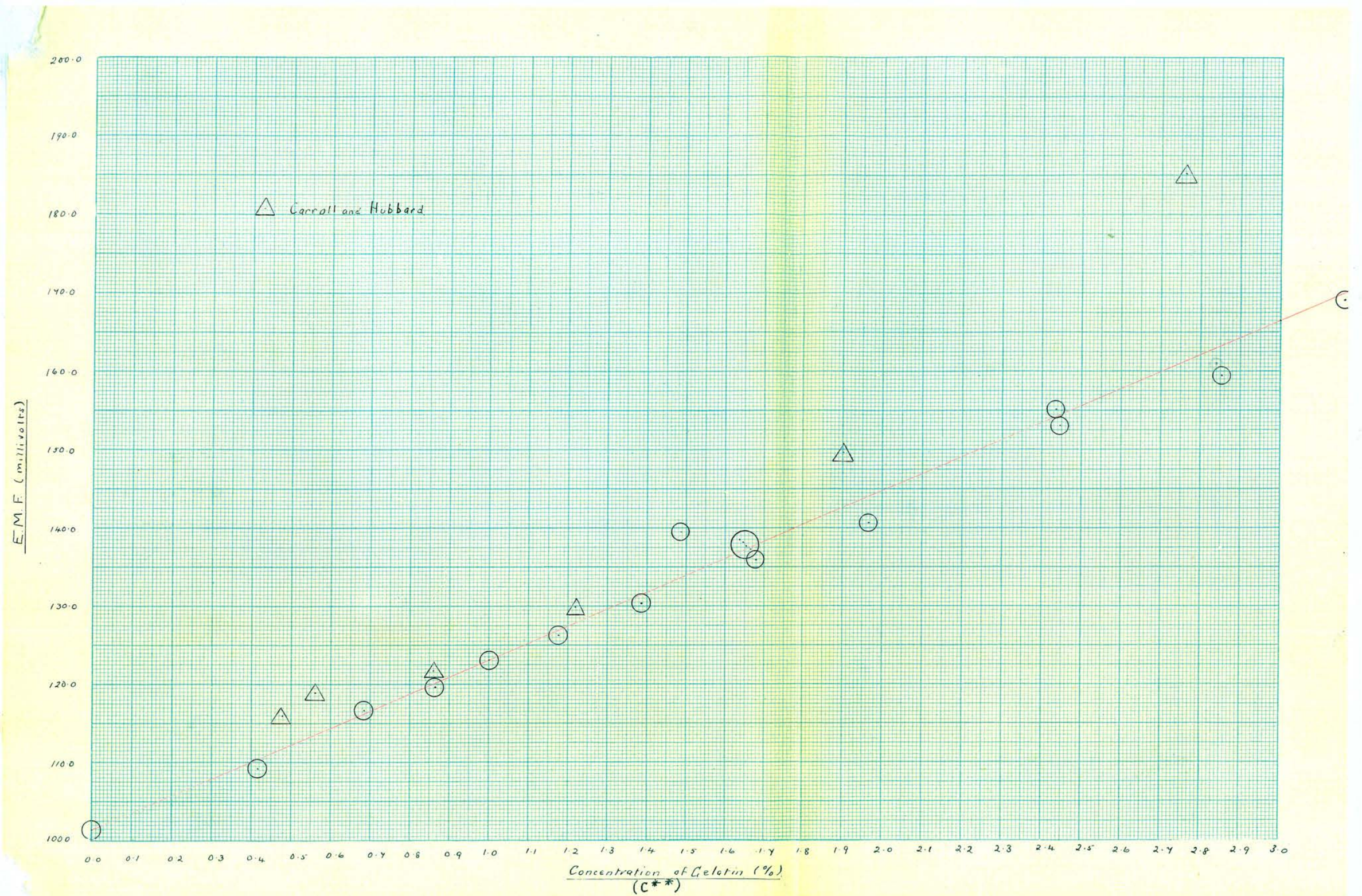
(1) See, for example, Linderström-Lang, Trans. Faraday Soc., 31 (1935) 324.
(2) Bolam and Donaldson, Trans. Faraday Soc., 29 (1933) 864.


200·0
190·0
180·0
170·0
160·0
150·0
140·0
130·0
120·0
110·0
100·0
E.M.F. (millivolts)
△ Carroll and Hubbard
0·0 0·1 0·2 0·3 0·4 0·5 0·6 0·7 0·8 0·9 1·0 1·1 1·2 1·3 1·4 1·5 1·6 1·7 1·8 1·9 2·0 2·1 2·2 2·3 2·4 2·5 2·6 2·7 2·8 2·9 3·0
Concentration of Gelatin (%)
(C**)

of free silver ion, derived as already described (p. 60), were calculated by means of the values of f_{Ag^+} given by Lewis and Randall.[1] Values for the amount of combined silver were obtained in the following manner.

Concentration combined Ag = Concentration $AgNO_3$ - Concentration free Ag.

Milli-equivalents Ag combined with one gm. of gelatin

$$= \frac{\text{Concentration combined Ag}}{\text{Concentration gelatin (\%)} \times 10}$$

The quantity C^{**} in the last column of Table XII was calculated by means of the formula:-

$$p + 10qC^{**} = 1.625,$$

where p = concentration of free silver, and q = the corresponding amount of silver combined with one gm. of gelatin, as given in the table. Thus C^{**} is the concentration of gelatin (%) which will reduce the free silver ion concentration in 1.625 m.e./l. silver nitrate to the value p, on the assumption that the amount of silver combined with unit weight of gelatin is independent of the gelatin concentration. In Figure V (p. 65) the values of C^{**} are plotted against the corresponding values of the e.m.f., and it is evident that the great majority of the points lie on or close to a straight line which cuts the y-axis at 101.4 milli-volts, the e.m.f. value for 1.625 m.e./l. silver nitrate in the

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(1) Lewis and Randall, loc. cit. It is questionable if the somewhat different values published by Kielland (J.A.C.S., 59 (1937) 1675) represent an improvement on those of Lewis and Randall.

absence of gelatin. The fact that the points lie on a common curve therefore fully confirms the view that, other things being equal, the amount of combination is independent of the gelatin concentration.

The equation to the straight line is

$$e = e_0 + kC^{**}$$

where e = the observed e.m.f., e_0 = e.m.f. in absence of gelatin, and k = constant. A survey of the available data shows that the results of other workers[1][2][3] conform to this relation. Values derived from Carroll and Hubbard's data,[4] obtained under the same conditions of temperature and pH as the author's, are plotted in Figure V, and show fair agreement with the present results.

A further example of the concordance between the e.m.f. and the precipitation data is afforded by the experiments (with stock gelatin at pH = 7.0) recorded in Table III (p. 21). In the first three of these experiments the concentrations of gelatin and silver nitrate were kept constant at 1.664 per cent. and 1.0 m.e./l. respectively, and estimation made of the concentration of the potassium chromate which just failed to produce precipitation in three hours. The required value was found to lie between 500 and 530

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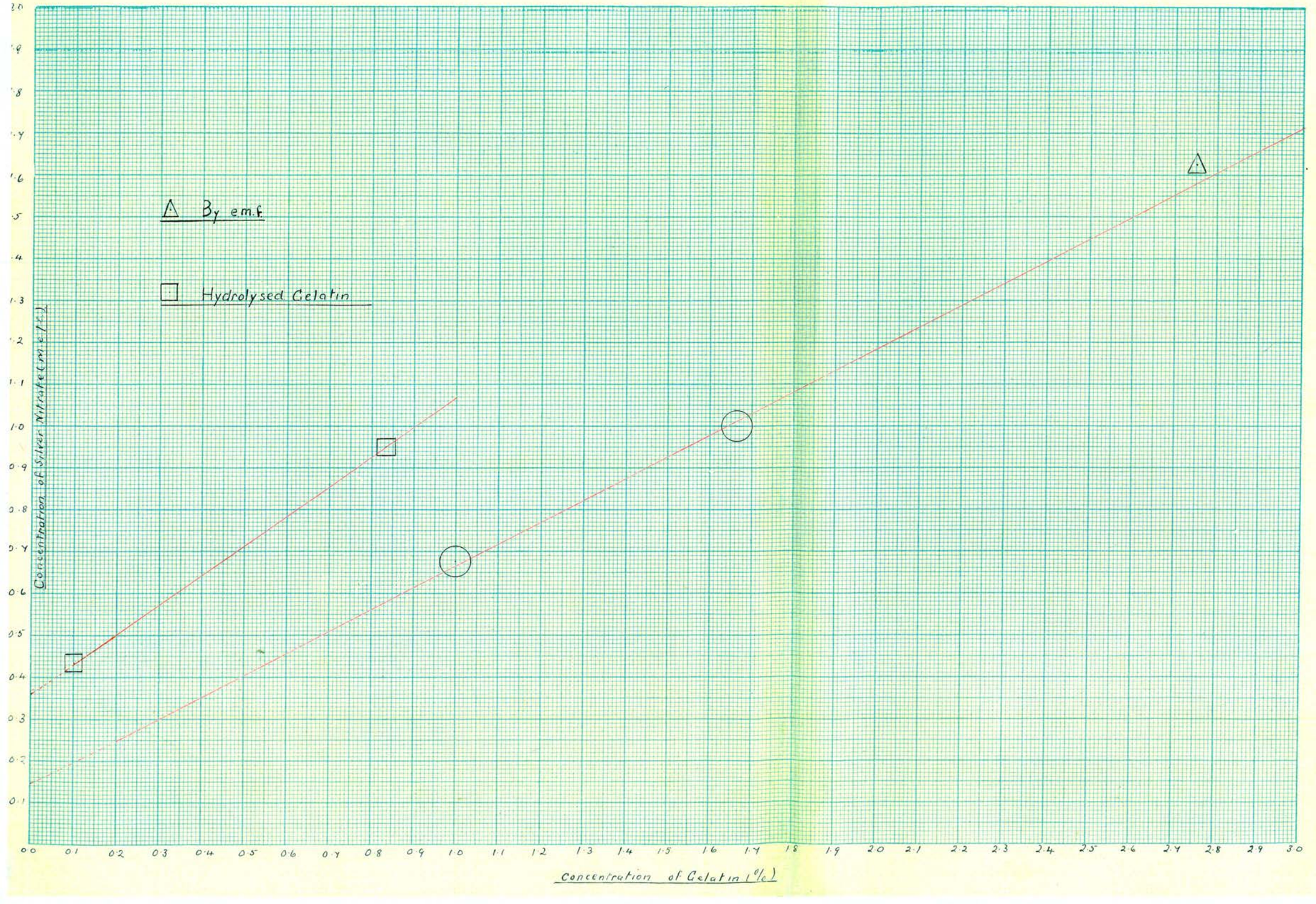
(1) Kruyt and Boelman, Koll. Beih., 35 (1932) 165.
(2) Bolam and Mackenzie, loc. cit.
(3) Bolam and Donaldson, loc. cit.
(4) Carroll and Hubbard, loc. cit. See, however, p.95.

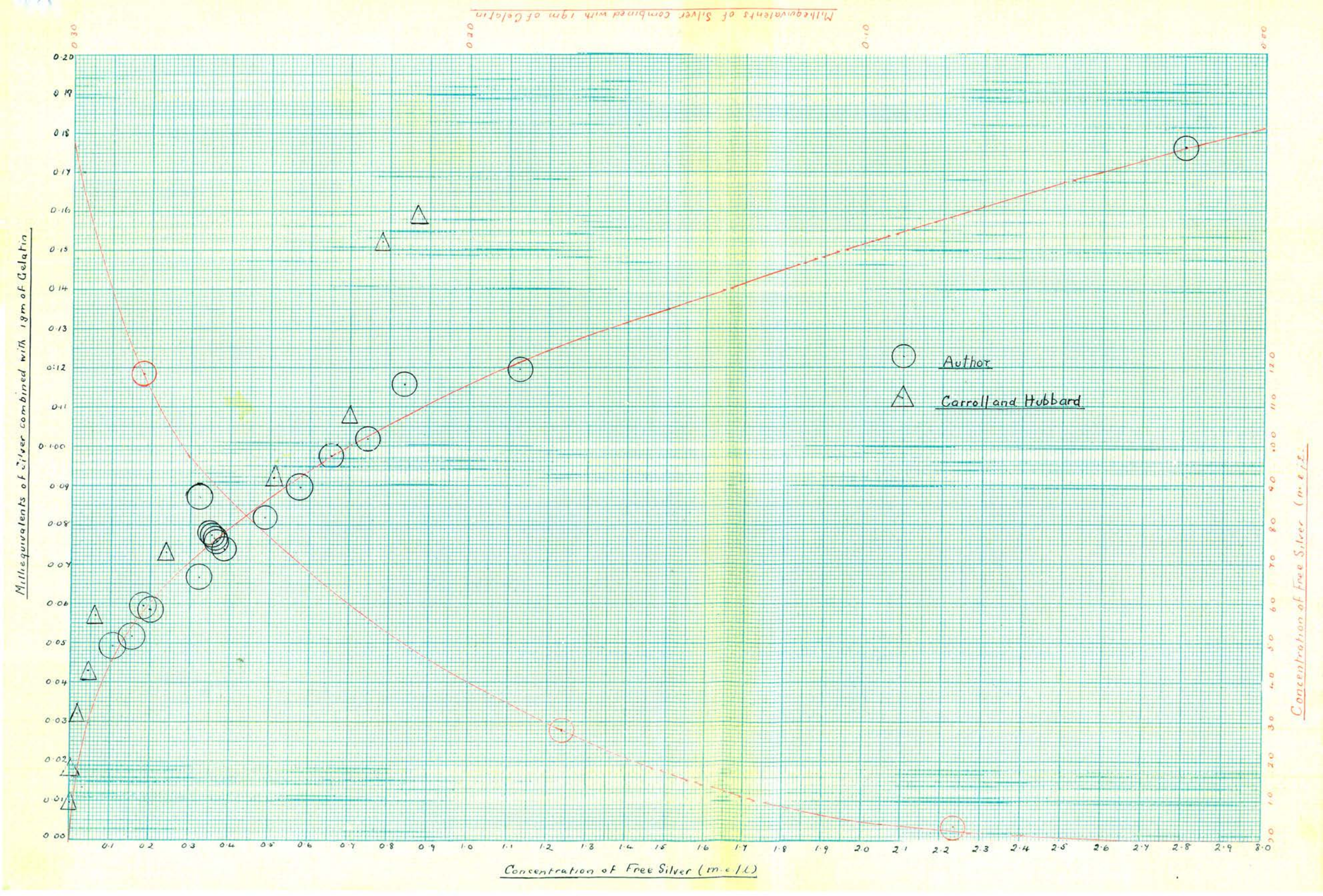
FIGURE VI


△ By e.m.f
□ Hydrolysed Gelatin
Concentration of Silver Nitrate (m.e./c.c.)
Concentration of Gelatin (%)

m.e./l., which means that in the presence of 1.664 per cent. gelatin and 515 m.e./l. potassium chromate, the threshold value of the silver nitrate was 1.0 m.e./l. for a delay in precipitation of three hours. In the other two experiments the threshold value was determined (in the usual way, i.e., by varying the silver nitrate) with 1% gelatin present, other conditions being the same as above. A mean value of 0.675 m.e./l. silver nitrate was obtained. (Table X, p. 56).

In Figure VI the two threshold values are plotted against the corresponding gelatin concentrations, and the linear portion of the straight line curve drawn as a straight line passing between the experimental points and distant from them by about the amount of permissible error. The line cuts the y-axis at 0.146 m.e./l., which is therefore the free silver ion concentration. Now the corresponding e.m.f. is 161.0 millivolts and by interpolation on the straight line in Figure V this value is also given by 1.625 m.e./l. silver nitrate containing 1.75% gelatin. 2·75 % Accordingly, this mixture should represent one of the points on the precipitation curve. By actual interpolation on the straight line in Figure VI, the gelatin concentration giving a threshold value of 1.625 n,e,/l., is found to be 1.83%, which is quite 2·83 %

FIGURE VII


Milliequivalents of Silver combined with 1gm of Gelatin
Concentration of Free Silver (m.e./L.)
Author
Carroll and Hubbard

close to the predicted value.

The Gelatin-Silver Ion Concentration Curve.

The relation between the combined and free silver in the case of the stock gelatin at pH = 7.0 is shown by the curves in Figure VII. Up to a free silver ion concentration of 3.0 m.e./l., the curve was drawn by means of values obtained by interpolation on the straight-line e.m.f. graph in Figure V. At higher concentrations the curve was drawn through the actual experimental points.* Carrol and Hubbard's data, which do not go beyond 1.0 m.e./l. free silver, are also included in the figure. It will be seen that up to about 0.7 m.e./l. free silver, the experimental points of Carroll and Hubbard lie on a curve similar to, but somewhat higher than that given by the present data. The two highest combination values obtained by these workers are obviously very much in error. Further consideration will be given to the combination curve at a later stage in the discussion.

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* Data in Table XII, p. 63.

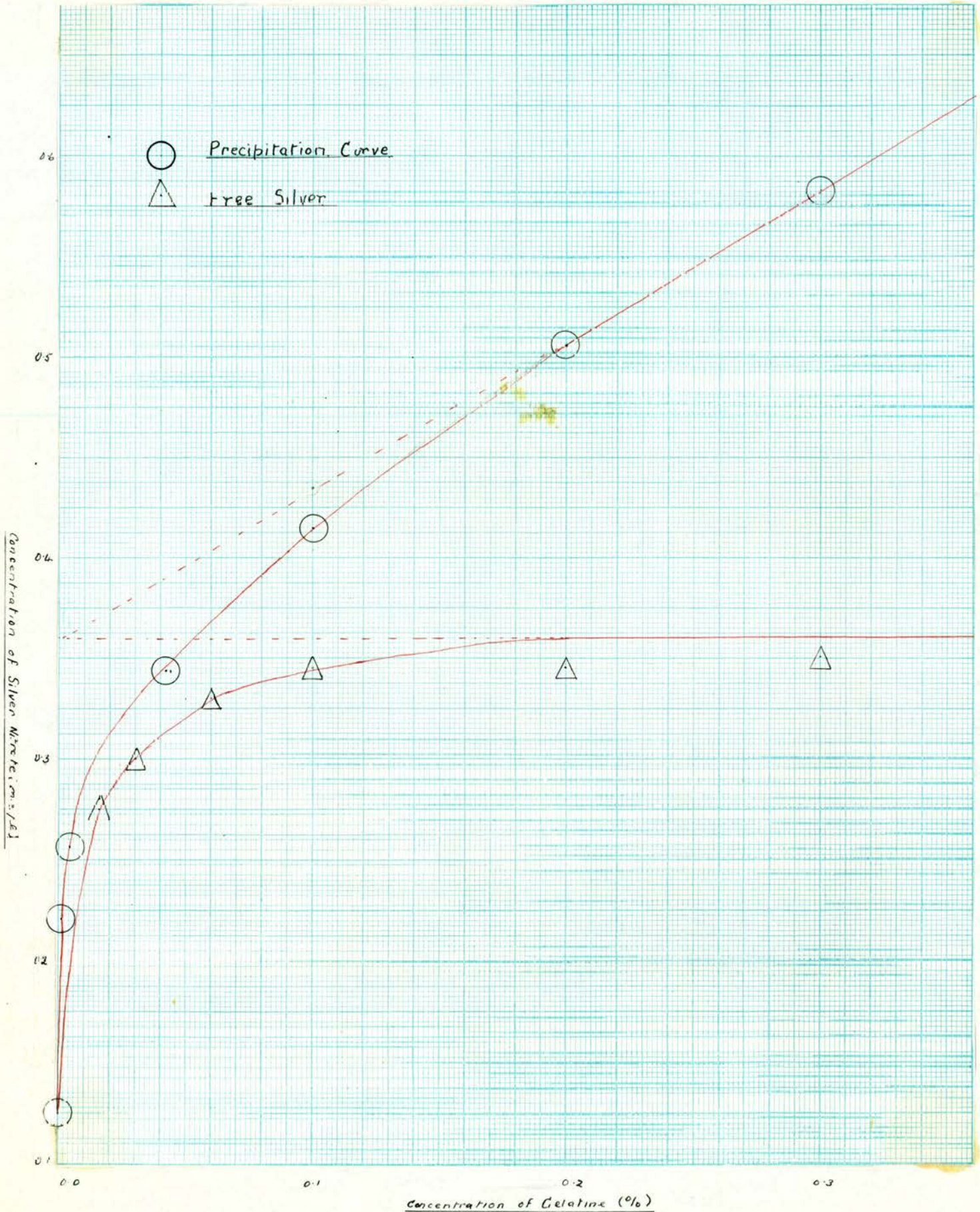
Precipitation Curve
Free Silver
0·6
0·5
0·4
0·3
0·2
0·1
0·0
0·1
0·2
0·3
Concentration of Silver Nitrate (mols/lt)
Concentration of Gelatine (%)

FIGURE VIII

The Inhibitive Action of Gelatin.

That portion of the precipitation curve for stock gelatin at pH = 7.0 below 0.35 per cent. gelatin is reproduced on a larger scale in Figure VIII. The lower curve in the figure shows how the concentration of free silver varied with increase in the gelatin concentration. Values for the free silver in mixtures containing less than about 0.2 per cent. gelatin could not be determined directly by measurement of the e.m.f., since the silver electrode behaved erratically when the concentrations of silver ion and gelatin were both low. In these cases therefore the free silver was deduced from the silver combination curve as follows. Consideration of the precipitation curve shows that the following relationship holds:-

Total conc. of silver = silver combined with one gm. of gelatin × conc. gelatin (%) × 10 + conc. of free silver.

From the combination curve may be obtained the amount of silver combined per gm. of gelatin at any particular concentration of free silver. By substituting corresponding values of free and combined silver in the above equation and varying the gelatin concentration, it is possible to arrive by a process of trial and error at a figure for the total concentration of silver which

lies sufficiently near the precipitation curve. This process was repeated for four different concentrations of free silver between 0 and 0.36 m.e./l., and the results, given below and plotted in Figure VIII, show how the free silver varied at low concentrations of gelatin.

Free Ag	Ag combined with 1 gm. gelatin.	Conc. gelatin (%)	Total conc. Silver.
0.275	0.0690	0.016	0.286
0.300	0.0710	0.030	0.321
0.330	0.0730	0.060	0.374
0.345	0.0750	0.100	0.420

In the absence of gelatin the threshold value of the silver nitrate, and therefore the concentration of the free silver ions, was 0.127 m.e./l., and with increasing gelatin concentration the concentrations of free silver rose to a maximum value of 0.36 m.e./l., i.e., almost a threefold increase. Since, as has been shown, the free chromate ion concentration must be practically constant over the linear portion of the precipitation curve, where the gelatin increases from 0.2 to 1.664 per cent., it follows that the proportion of chromate combined with the gelatin must be small, so that the free chromate ion concentration may be regarded without serious error as having a constant

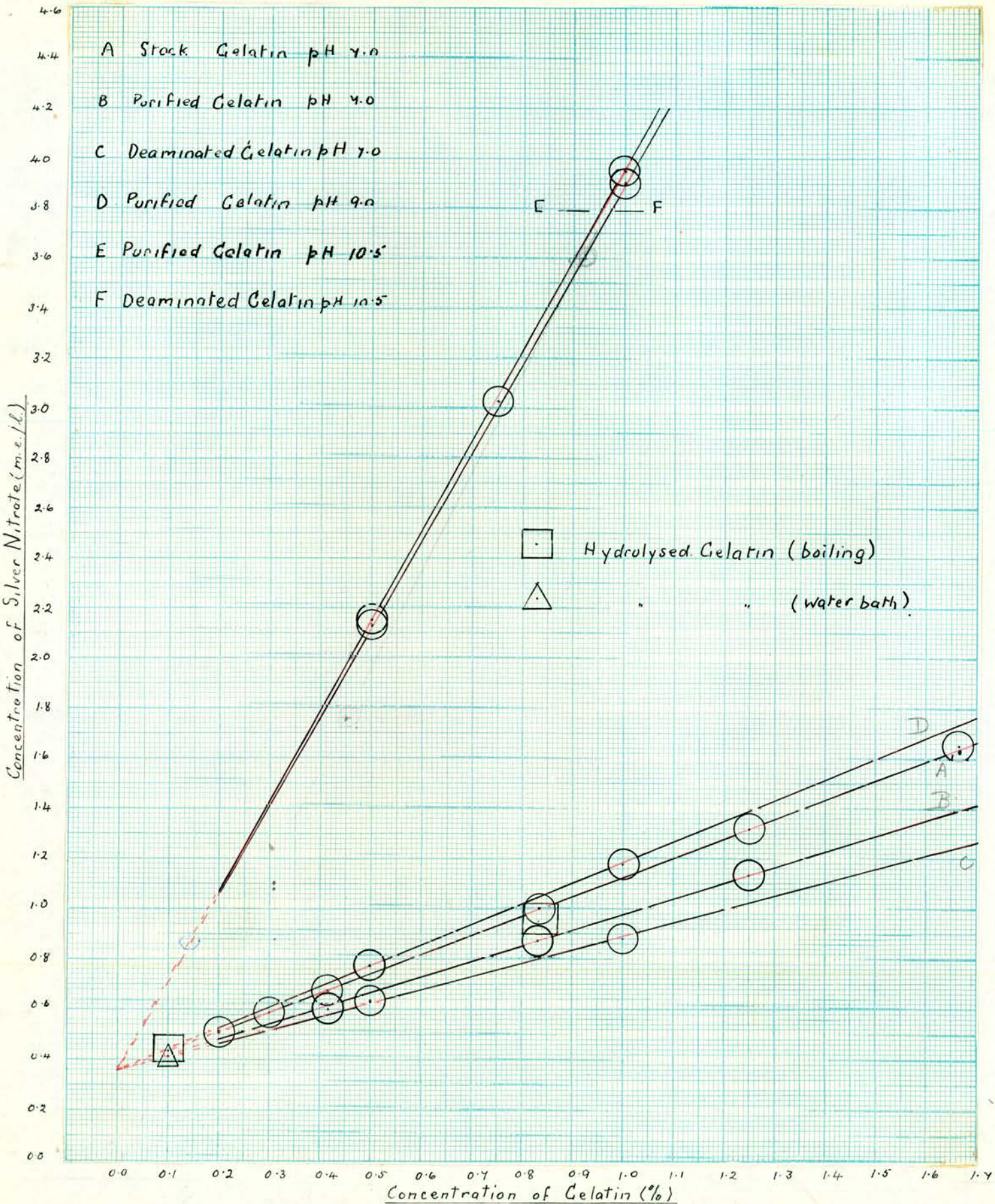

FIGURE IX

PRECIPITATION CURVES.

value in the neighbourhood of 25.0 m.e./l. over the entire curve. Hence it must be concluded that the increase in free silver ion is not due to a decrease in the free chromate ion, but results from some action of the gelatin which inhibits the formation of solid silver chromate from a supersaturated solution of the salt.

That the maximum degree of supersaturation was unaffected by increasing the pH, up to 10.5, or by hydrolysis, purification or deamination of the gelatin is shown by the precipitation curves in Figure IX.* The appropriate data are given in detail in Tables IV, V, and VI (pp. 22 - 25) and the most probable values for the threshold concentrations are collected in Tables X (p. 56) and XIII (p. 77). In every case the experimental points lie on a straight line which cuts the y-axis at 0.36 m.e./l. Thus the maximum value of the free silver ion concentration, and therefore the maximum degree of supersaturation, must be the same in all cases. This result supports the view that the inhibition is due to the gelatin itself, rather than to degradation products or impurities arising out of the method of manufacture.

The question arises as to whether the concentration of gelatin at which the degree of supersaturation reaches its maximum value varies from case to case,

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* The precipitation curve for boiled gelatin is shown separately in Figure VI.

TABLE XIII.

Threshold Values of Silver Nitrate.

Gelatin	pH	Conc. gelatin (%)	Threshold value silver nitrate (m.e./l.)
Purified	7.0	0.416	0.600
	"	0.832	0.875
	"	1.250	1.133
	9.0	0.500	0.775
	"	1.000	1.175
	10.5	0.500	2.150
	"	0.750	3.030
	"	1.000	3.950
Deaminated	7.0	0.500	0.630
	"	1.000	0.880
	10.5	0.500	2.130
	"	1.000	3.900

although the maximum degree of supersaturation itself remains the same. Comparison of the data for the untreated stock gelatin with that for boiled gelatin indicates that such variation may occur. As the precipitation curve for the hydrolysed gelatin (Fig. VI) shows, the threshold values at 0.1 and 0.832 per cent. gelatin lie on a straight line cutting the y-axis at 0.36 m.e./l. Thus the maximum degree of supersaturation is attained at or before 0.1 per cent. gelatin, whereas in the case of the untreated gelatin (Fig.VIII) the corresponding value is 0.2%. The effect appears to be real, since the threshold values (Table X) at 0.1 per cent. gelatin are 0.415 m.e./l for the untreated material, and 0.435 m.e./l for the hydrolysed, a difference of 5%, which is well outside the usual experimental error. It would seem that the apparent loss in the imhibitive power of the gelatin on hydrolysis reported by Bolam and Desai[1] and Desai and Nabar,[2] was probably due to increase in the free chromate ion concentration, since Donaldson[3] observed that the combination of chromate with gelatin decreased with hydrolysis of the protein.

The formation of the solid phase from supersaturated solutions of sparingly soluble salts is a complicated process. Under suitable conditions three stages at

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(1) Bolam and Desai, Trans. Faraday Soc., 24 (1928) 50.
(2) Desai and Nabar, Trans. Faraday Soc., 28 (1932) 449.
(3) Donaldson, Thesis (Edinburgh) 1933.

least may be distinguished, as follows: Initially there is an interval of time, the "induction period", during which no visible change occurs and the concentration of the solution remains practically constant. This is followed by a second stage, characterised by the sudden appearance of the solid salt and a rapid decrease in the concentration. The succeeding and final stage consists in the further slow separation of the solid salt, most conveniently detected by the accompanying gradual decrease in the concentration of the solution. These effects are well illustrated by the recent investigations of Jensen[1] on calcium fluoride, Reitemeier and Buehrer[2] on calcium carbonate, and Van Hook[3] on silver chromate.

It is generally accepted that the main process occurring during the induction period is the formation of stable crystal nuclei, and that the later stages are concerned chiefly with the growth of these nuclei, this taking place rapidly during the second stage and much more slowly in the third. To be stable, a nucleus must attain a certain minimum size, otherwise it tends to disintegrate, as shown by the high solubility of very small particles. Volmer[4] has pointed out that it is very improbable that the formation of a stable nucleus

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(1) Jensen, Zeitschr. phys. Chem., 180A (1937) 93.
(2) Reitemeier and Buehrer, J. Phys. Chem., 44 (1940) 535, 552.
(3) Van Hook, J. Phys. Chem., 44 (1940) 751.
(4) Volmer, Zeitschr. Elektrochem., 35 (1929) 557.

occurs through the simultaneous aggregation of the number of ions necessary to give a particle of the required dimensions. Rather, the process is to be regarded as an unusual type of growth, in which the nuclei are alternately waxing and waning, a proportion of them reaching the critical size as a result of favourable fluctuations in the ionic density. Jensen (loc. cit.) agrees that the formation of stable nuclei is a growth process but appears to take the view that the potential nuclei steadily increase in size throughout the induction period.

Since precipitation occurs almost immediately when solutions containing 0.36 m.e./l. silver nitrate and 25.0 m.e./l. potassium chromate are mixed in the absence of gelatin, it is evident that one of the effects of the gelatin is greatly to increase the length of the induction period. Hence, the gelatin must oppose the formation of stable nuclei. The early suggestion made by Tryhorn and Blacktin[1] and by Bogue,[2] that the gelatin is adsorbed on the reacting ions and so prevents them from coming into contact is hardly feasible. It is, however, highly probable that the gelatin is adsorbed by the potential crystal nuclei and so reduces the rate of their growth by opposing the further accretion of ions. This would be in harmony with the initial continuous

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(1) Tryhorn and Blacktin, Trans. Faraday Soc., 19 (1923) 433.
(2) Bogue "The Chemistry and Technology of Gelatin and Glue", (1922) 563.

increase in the degree of supersaturation with increase in the gelatin concentration, since the amount of adsorption would also increase. Moreover, since there will be an upper limit to the adsorption, it is to be expected that the inhibiting action of the gelatin will not increase indefinitely, but reach a maximum value at some low concentration of the protein, as actually happens.(1)

The inhibitive action of the gelatin would thus appear to resemble its ordinary "protective action" in the sense that the protein forms a sheath round the incipient nuclei. However, the concentration of the gelatin at which the maximum inhibitive effect is just attained is of a very different order of magnitude from the minimum concentration required for the protective action. Thus the concentrations corresponding to the "gold numbers" which are commonly found in the literature(2) vary from about 0.0005 per cent. to 0.0025 per cent., as compared with 0.2 per cent., the concentration of stock gelatin at which the maximum inhibitive action was reached.

Van Hook (loc. cit.) has derived the following expression from a consideration of the kinetics of nuclei formation.

$$JS_0 = J(H_0 - K_{SP}) = \text{constant} = k$$

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(1) That gelatin is adsorbed by ionic crystals has been shown by several workers. See, for example, Miles, Phil. Trans. Royal Soc., A235 (1935) 160.

(2) For references, see Clayton, "Colloid Aspects of Food Chemistry and Technology" (1932) 68, 69.

where J = the induction period, S_0 = supersaturation, H_0 = ion product, and K_{SP} = solubility product. The equation was found to hold for the precipitation of silver chromate from aqueous solution ($H_0 = [Ag^+]^2[CrO_4^=]$), provided the values of H_0 and of the ratio R = equivalents $AgNO_3$/equivalents K_2CrO_4, were kept within certain limits. Under these conditions the value of k was 0.01×10^{-7}, when J was expressed in minutes.

For the experiments with stock gelatin in which 25.0 m.e./l. potassium chromate was present, the relevant data are: J = 120 minutes, $[Ag^+] = 0.36 \times 10^{-3}$ mol/litre, $[CrO_4^=] = 12.5 \times 10^{-3}$ mol/litre, $H_0 = 1.62 \times 10^{-9}$, R = 0.014, and K_{SP} may be neglected. The value of k is therefore 1.94×10^{-7}, so that, according to the formula, when the chromate concentration was 515 m.e./l., (in which case $[Ag^+] = 0.146 \times 10^{-3}$ mol/litre, $[CrO_4^=] = 258 \times 10^{-3}$ mol/litre, $H_0 = 5.48 \times 10^{-9}$, R = 0.0003) the value of J should have been 34 minutes. Actually, J increased to 180 minutes with increase in the chromate concentration, i.e., with decrease in R. In contrast with this result, Van Hook reports that when R was decreased below the lower limit for constancy of k, the observed value of J was less than the calculated value.

A considerable amount of time was spent in attempts to devise a satisfactory method of studying the course of the precipitation by means of the ultramicroscope. The work, however, proved unexpectedly difficult, and was

discontinued, as it was desired to investigate the problem of the combination of gelatin with silver ions.

The Combination of Gelatin with Alkali.

In the last column of Table I (page 12) are shown the volumes (ml.) of 0·02 N. sodium hydroxide or 0·01 N. hydrochloric acid required to bring the pH of 10 ml. of a 1·0% solution of gelatin to the given values. The amounts of alkali combined with the protein under these conditions were calculated in the following manner, as illustrated by the case of purified gelatin at pH = 9·0.

Volume 0·02 N. NaOH required to bring pH of 10 ml. of 1·0% gelatin from 4 ·85 - 4·90 to 9·0 = 1.76 ml.

Total concentration added alkali = $\frac{1{\cdot}76 \times 0{\cdot}02}{10}$ = 3·52 m.e./l.

Concentration of free alkali = $10^{-14/9}$ = 0·01 m.e./l.

Concentration of combined alkali = 3·52 - 0·01 = 3·51 m.e./l.

Hence, alkali combined with 1 gm. gelatin = 3·51/10 = 0·351 milli-equivalent.

Volume 0·01 N. HCl required to bring pH of 10 ml. of 1·0% gelatin from 4·85 - 4·90 to 4·70 (iso-electric point) = 0·48 ml.

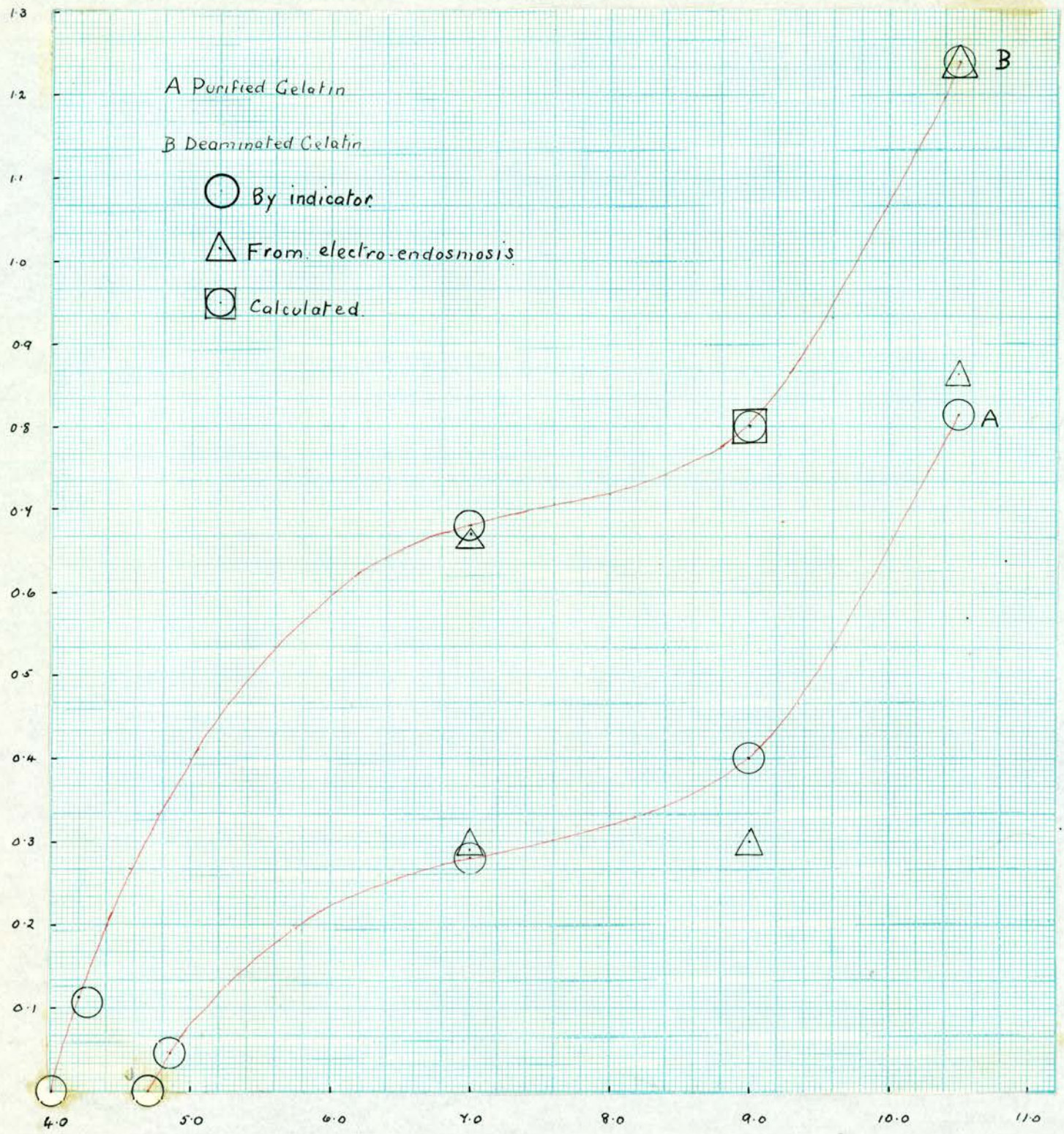
A Purified Gelatin
B Deaminated Gelatin
By indicator
From electro-endosmosis
Calculated
A
B
Milli-equivalents alkali combined with 1 gm. gelatin
1·3
1·2
1·1
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
4·0
5·0
6·0
7·0
8·0
9·0
10·0
11·0
pH

FIGURE X

Total concentration acid = $\frac{0{\cdot}48 \times 0{\cdot}01}{10}$ = 0·48 m.e./l.

Concentration free acid = antilog -4·7 = 0·02 m.e./l.

Concentration neutralised acid = 0·48 - 0·02 = 0·46 m.e./l.

Hence acid neutralised by 1 gm. gelatin = 0·46/10 = 0·046 milli-equivalent.

Thus, at pH = 9·0, alkali combined with 1 gm. originally iso-electric gelatin = 0·351 + 0·046 = 0·397 milli-equiv., (i.e., combined alkali = 0·397 m.e./g.).

The values of combined alkali, obtained in the above fashion for purified and deaminated gelatin are given in the third column of Table XIV (p. 86), and plotted against pH in Figure X. The iso-electric point of the deaminated material was assumed to lie at pH = 4·0.

In the fourth column of Table XIV are shown values for the combined alkali derived from the electro-endosmotic data, on the assumption that the rate of flow was directly proportional to the amount of combined alkali, by means of the formula:-

Milli-equivalents alkali combined with 1 gm. gelatin = rate of flow × 0·033.

The rates of flow, calculated in the manner already described (p. 48), are given in Table XV (p. 87).

It will be seen from Table XIV and Figure X that

TABLE XIV.

Combination of Gelatin with Alkali.

Gelatin	pH	Milli-equiv. alkali combined with 1 gm. gelatin.	
		Indicator	Electro-endosmosis
Purified	4.85-4.9	0.046	-
	7.0	0.282	0.290
	9.0	0.397	0.297
	10.5	0.815	0.875
Deaminated	4.2	0.117	-
	7.0	0.679	0.677
	9.0	(0.797)	-
	10.5	1.243	1.241

TABLE XV.

Rate of Electro-endosmotic Flow.

Gelatin	pH	Conc. $AgNO_3$ (m.e./l.)	Ionic strength	Time of flow (secs.)	Rate of flow. (μ/sec/volt/cm.)
Purified	7.0	-	23.66	359.0	8.80
	"	1.58	23.66	519.0	6.10
	9.0	-	23.66	350.0	9.00
	"	2.01	23.65	562.0	5.61
	10.5	-	23.66	119.0	26.50
	"	7.64	23.66	231.5	13.60
Deaminated	7.0	-	29.49	154.0	20.50
	"	1.34	29.49	155.0	20.40
	10.5	-	29.34	84.0	37.60
	"	7.44	29.54	85.0	37.15

the two series of values for combined alkali are in good agreement, except in the case of purified gelatin at pH = 9·0.

As originally defined, electro-endosmosis denotes the movement of a liquid along the surface of a fixed solid under the influence of a potential gradient in the direct of the interface. It is frequently postulated[1] that the rigidity of a gelatin gel is due to the linking up of molecules or particles of the protein to form a continuous framework, the interstices of which are filled with liquid. Jordan Lloyd[2] has advanced evidence in support of the view that the framework is composed of insoluble iso-electric or neutral gelatin, any electrically charged gelatin being dissolved in the liquid phase. With regard to the electro-endosmotic flow of aqueous solutions through the gel, it would appear immaterial whether the charged gelatin is incorporated in the framework or present in the solution. In either case the movement of the charged portions of the protein is severely restricted, since, on the one hand, the framework possesses considerable rigidity, and, on the other, dissolved gelatin cannot escape from the cavities in the gel. Hence the essential condition for electro-endosmosis, namely, the presence of relatively fixed charges in contact with

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(1) Jordan Lloyd and Shore, "Chemistry of the Proteins" (1938) 481.

(2) Jordan Lloyd, Biochem. J., 14, 147, 584. Jordan Lloyd and Shore, loc. cit., pages 397 and 481

freely mobile water, will be realised in either case.

According to the accepted theory of electro-endosmosis,[2] the rate of flow of the liquid varies in direct proportion to the density of the electrical charge upon the solid wall, provided other factors (including the ionic strength in the case of solutions) are kept constant. Hence the simplest interpretation of the proportionality between the values for combined alkali obtained as described above, and the rates of endosmotic flow, is that the combination of a given amount of the alkali with the gelatin produced an equivalent increase in the negative charge on the protein. More precisely, the negative charge on the gelatin increased by one electron for each hydroxyl ion with which the gelatin reacted.

The only other investigation of the electro-endosmotic flow in gelatin gels comparable with the present, is that of Ghosh,[1] who found that over the pH range 2·10 - 3·62 the water moved to the anode at a rate approximately directly proportional to the amount of hydrogen ion combined with the protein, as determined by the e.m.f. method. In the present work it was also observed that the water flowed to the anode when the gel was brought to the acid side of the iso-electric point by the addition of hydrochloric acid. Abramson[2] has

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(1) Ghosh, J.C.S., 1928, 712.
(2) Abramson, "Electrokinetic Phenomena" (1934) 155.
J. Gen. Physiol., 15 (1932) 575.

examined the electrophoresis of quartz particles suspended in solutions of ordinary and deaminated gelatin over the pH range 2·9 - 5·9. He found that in both cases the velocity of migration was directly proportional to the amount of combined acid or alkali, as determined by electrometric titration. All these results are in harmony with the above interpretation of the electro-endosmotic data obtained in the present research.

The iso-electric points of the gelatins were not established by experiment. That for the purified gelatin was assumed to be 4·7, since this value is frequently quoted in the literature. The adopted value of 4·0 for the deaminated material was that obtained by Hitchcock[1] (minimum osmotic pressure) and Loebel[2] (minimum combination with dyes and minimum viscosity).[3] While these values lead to good agreement between the combination and electro-endosmosis data, it is realised that they may not be quite correct in the present instance. Evidence is accumulating to show that the iso-electric point varies from case to case, depending upon the method employed in converting the collagen to gelatin[4][5], and it would appear that the iso-electric point may lie between 4·7 and 5·0 or between 6·0 and 8·0.

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(1) Hitchcock, J. Gen. Physiol., 6 (1923-24) 95. Quoted by Jordan Lloyd and Shore (loc. cit.), page 316.
(2) Loebel, J. Phys. Chem., 32 (1928) 763.
(3) Abramson (loc. cit.) obtained 3·9 by electrophoresis.
(4) Briefer, J. Ind. Eng. Chem., 21 (1929) 266.
(5) Hunter and Turner, Trans. Faraday Soc., 36 (1940)835.

It is certain, however, that the iso-electric point of the gelatin employed in the present work fell within the lower range of pH.

In order to compare the values for combined alkali with the data obtained by others, Table XVI (p. 92) has been compiled from the titration curves (for undeaminated gelatin) published by various workers. It was assumed that the iso-electric point was 4·7 in all cases. This approximation appeared justifiable, in the absence of evidence to the contrary, except in the case of the data of Hunter and Turner, who obtained values ranging from 4·9 to 5·1 from electrophoretic and other observations. On the basis of these values, the combined alkali is much lower, namely, 0·21 and 0·20 m.e./g.

TABLE XVI./

TABLE XVI.

Reference	Temp.	Alkali (m.e./g.) combined at pH		
		7·0	9·0	10·5
1	(?)	0·36	0·44	0·63
2	25°	0·30	0·35	0·51
"	40°	0·30	0·39	0·69
3	25°	0·27	0·34	0·57
4	25°	0·26	0·34	0·50
5	Room	0·29	0·35	0·47
6	30°	0·35	0·44	0·73
7	25°	0·32	0·47	-
8	30°	0·32	0·40	0·61
9	35°	0·29	-	-
"	"	0·26	-	-

1: Loeb, J. Gen. Physiol., 3 (1920-21) 85.
2. Hitchcock, J. Gen. Physiol., 6 (1923-24) 457.
3. Atkin and Douglas, J. Soc. L.T. Chem., (1924). Quoted by Jordan Lloyd and Shore (loc. cit.) p. 309.
4. Simms, J. Gen. Physiol., 11 (1927-28) 629.
5. Loebel, J. Phys. Chem., 32 (1928) 763.
6. Hitchcock, J. Gen. Physiol., 15 (1931) 125.
7. Carroll and Hubbard, loc. cit.
8. Ehrenberg and Wulff, Kolloid. Beih., 42 (1935) 8.
9. Hunter and Turner, loc. cit.

It is evident that the amounts of alkali combined at pH = 7·0 and pH = 9·0 found by the author (by the colorimetric method) are much the same as those observed by the majority of the other workers. At pH = 10·5, however, the present value is considerably higher than any of the values shown in Table XVI. In view of the concordance between the colorimetric and electro-endosmotic data, it would seem that the difference is real, and not the result of error due to some action of the protein upon the indicator, or to interference by the carbon dioxide of the atmosphere.

The titration curve was found to be displaced by deamination in much the same fashion as was observed by Loebel (loc. cit.), but Simms (loc. cit.), employing dialysed deaminated gelatin, obtained curves which converge over the pH range 9·0 - 12·0. Loebel concludes that the combining capacity of the protein for alkali is increased by the formation of acidic hydroxyl groups as the result of the treatment with nitrous acid. On the other hand, it would appear from the data of Simms that the maximum amount of alkali combined remains the same. In any case, however, it is evident that some displacement of the titration curve must occur, apart from any change in the combining capacity of the gelatin.

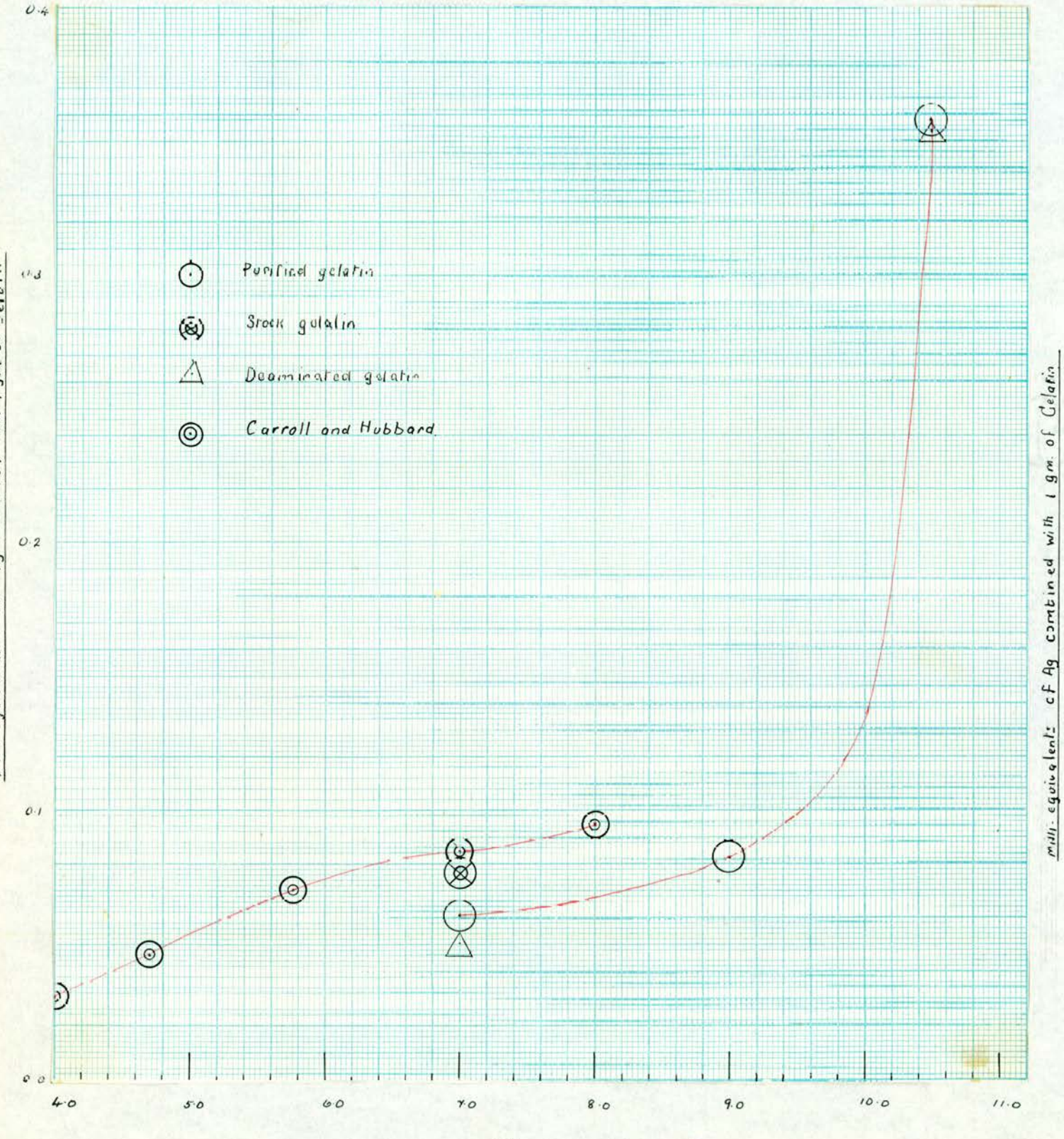
0.4
0.3
0.2
0.1
0.0
Purified gelatin
Stock gelatin
Deaminated gelatin
Carroll and Hubbard
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
pH
Milli-equivalents of Ag combined with 1 gm of Gelatin
Milli-equivalents of Ag combined with 1 gm. of Gelatin

FIGURE XI

The Combination of Gelatin with Silver Ions.

The data in Table XVII (p. 96) show how the combination of the gelatin with the silver varied with the treatment of the gelatin and the pH of the solution, the concentration of free silver being kept constant at 0·360 m.e./l. In the last column of the table are values for the amounts of combined silver, as derived from the precipitation curves (Fig. IX, page 75) by means of the formula on page 59. The data are also plotted in Figure XI, and it will be seen that the combination was (a) appreciably reduced by the purification of the gelatin, (b) practically unaffected by deamination, (c) slightly increased by raising the pH from 7·0 to 9·0, and (d) markedly increased by raising the pH from 9·0 to 10·5.

The silver combination curve of Carroll and Hubbard for the free silver ion concentration of 0·35 m.e./l. is also shown in Figure XI. Although Carroll and Hubbard employed purified gelatin, their curve does not link up with the author's curve for such gelatin, but lies considerably higher, showing, indeed, better agreement with the single point obtained with stock gelatin at pH = 7·0. However, there is no reason to doubt that the combination with silver is greater at pH = 7·0 than at the iso-electric point. This is indicated not only by the results of Carroll and

TABLE XVII.

Combination of Gelatin with Silver.

Gelatin (1%)	pH	Total Silver (m.e./l.)	Free Ag^+ (m.e./l.)	Milli-equiv. silver combined with 1gm. gelatin
Stock	7.0	1.122	0.360	0.076
Purified	7.0	0.970	0.360	0.061
	9.0	1.180	0.360	0.082
	10.5	3.940	0.360	0.358
Deaminated	7.0	0.870	0.360	0.051
	10.5	3.900	0.360	0.354

Hubbard, but also by those of Ehrenberg and Wulff,[1] who observed that when solutions of purified gelatin were shaken with excess of silver chloride until equilibrium was reached, the concentration of dissolved silver increased continuously with increase in the alkalinity of the solution.

On comparing Figure XI with Figure X (p. 84), it is evident that the combination with silver and that with alkali vary in much the same way with variation in the pH. In both cases the curve is S-shaped, and the inflections occur in the regions of pH = 6·0 and 9·0.[2]

Carroll and Hubbard conclude that "the silver is probably attached to the amino groups of the gelatin", mainly on the ground that this would account for the absence of combination in the presence of ammonia. They remark that "if this theory is correct, there should be no selective combination of the $Ag(NH_3)_2^+$ ion with gelatin, since the affinity of the silver for amino nitrogen is satisfied". Carroll and Hubbard further suggest that the increase in combination of silver with gelatin which accompanies increase in the pH may be ascribed to the opening of peptide linkages, by the alkali, to give free amino groups, thus:-

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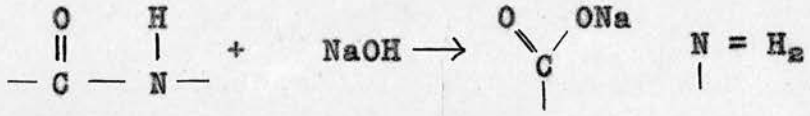
(1) Ehrenberg and Wulff, Kolloid. Beih., 42 (1935) 1.
(2) Goigner and Pauli (Biochem. Z., 235 (1931) 271) found that when a solution of iso-electric gelatin was shaken with excess of silver oxide until equilibrium was reached, the proportion of combined silver was much greater than in a similar gelatin solution containing the same total amount of silver as silver nitrate. This further illustrates the increase in combined silver with increase in the alkalinity.



Commenting on Carroll and Hubbard's view that the silver ion combines with the free amino groups of the protein molecule, Schmidt,(1) however, points out that "in line with the Zwitterion concept the number of $-NH_2$ groups should increase with increasing pH and hence increasing amounts of silver should be bound".

In the absence of evidence to the contrary, it would be reasonable to suppose that the silver ions form complexes with the NH_2-groups of the gelatin, since Northrop and Kunitz(2) have shown that in all likelihood cupric ions form such complexes, and since silver ions resemble those of copper in readily forming complex ions with molecules of ammonia. However, the fact, established by the present work, that deamination of the gelatin has little, if any, influence upon the amount of silver combined, does not accord with the view that the silver combines with the NH_2-groups, whether these arise in the manner indicated by Schmidt, or according to the process described by Carroll and Hubbard.

The results of various workers strongly indicate that the main effect of the action of nitrous acid

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(1) Schmidt, loc. cit., p. 696.

(2) Northrop and Künitz, J. Gen. Physiol., 11 (1927-28) 481.

upon gelatin is the removal of the (free) ϵ -amino groups of the lysine residue in the protein. Thus, (a) on treatment of gelatin with nitrous acid, no lysine can be isolated after acid hydrolysis;[1] (b) gelatin treated with nitrous acid in the Van Slyke apparatus yields one half as much nitrogen as is found in the lysine fraction after acid hydrolysis of the original gelatin;[2][3] (c) on treatment with nitrous acid, the decrease in total nitrogen is equal to the nitrogen evolved from untreated gelatin in the Van Slyke apparatus;[3][4] (d) if gelatin, previously benzenesulphonated, is hydrolysed with formic and sulphonic acids, at least 50 per cent. of the nitrogen lost on treatment with nitrous acid can be accounted for by the isolation of ϵ -aminobenzene-sulphonyl-d-lysine;[5] (e) the bromine content of the compound formed by the action of p-bromophenyl isocyanate on gelatin usually represents 70 to 90% of the lysine content of the protein.[6]

According to Schmidt, Kirk and Appleman,[7] in the

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(1) Skraup and Kaas, Ann., 351 (1906) 379.
(2) Van Slyke and Birchard, J. Biol. Chem., 16 (1913-14) 539. Dakin, J. Biol. Chem., 44 (1920) 499. Northrop, J. Gen. Physiol., 3 (1920-21) 715. Albanese, J. Biol. Chem., 134 (1940) 467.
(3) Hitchcock, J. Gen. Physiol., 6 (1923-24) 95.
(4) Loebel, J. Phys. Chem., 32 (1928) 763.
(5) Gurin and Clarke, J. Biol. Chem., 107 (1934) 395.
(6) Hopkins and Wormall, Biochem. J., 27 (1933) 740, 1706; Nature, 134 (1934) 290.
(7) Schmidt, Kirk and Appleman, J. Biol. Chem., 88 (1930) 285. Quoted by Jordan Lloyd and Shore, "Chemistry of the Proteins" (1938) 307, and Hitchcock, in Schmidt, "The Chemistry of the Amino Acids and Proteins" (1938) 613.

case of lysine itself, the value of pK for the dissociation of the ϵ-amino group is 10·53 at 25°.[1] Hence, since the ϵ-amino group of the lysine in proteins is presumably situated far from other groups, it may be expected to dissociate in the region of pH = 10·5,[2] and the form of the titration curve in a number of cases is actually consistent with this. Thus Cohn, Green and Blanchard[3] obtained pK = 10·8 for horse carboxyhæmoglobin, Green[2] pK = 10·5 - 10·7 for normal horse serum globulins, and Simms[1] pG = 10·4 - 10·6 for gelatin.

In the light of the foregoing it would be natural to connect the rapid increase in the combination of gelatin with silver which commences at pH = about 9·0, with the formation of unionised ϵ-amino groups. Since, however, treatment of the gelatin with nitrous acid does not result in any decrease in the amount of combined silver, it must be concluded that the silver ions are not attached to these amino groups, or, for that matter, to any other amino groups which may be present.

As Carroll and Hubbard point out, another possibility is the formation of a silver gelatinate entirely analogous to the sodium gelatinate of Loeb's theory,

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(1) Levene and Simms (see Simms, J. Gen. Physiol., 11 (1927-28) 629) obtained the very similar value of 10·45 for the "titration index", pG.
(2) Green, J.A.C.S., 60 (1938) 1108. Jordan Lloyd and Shore (loc. cit.) pp. 303-305.
(3) Cohn, Green and Blanchard, J.A.C.S., 59 (1937) 509.

but distinguished from it by insolubility. Carroll and Hubbard consider that the coagulation of proteins by silver salts is evidence for the view, and that the gelatinate would be subject to hydrolysis decreasing with increasing alkalinity. However, these workers conclude that their own experimental data do not support the view in question, as will be seen from the following quotation from their paper (p. 820): "At constant pH and $[Ag^+]$, the combined silver per gram of gelatin was independent of gelatin concentration, since all the data for pH 7 fall on the same curve within the limits of error. This permits us to eliminate the hypothesis of an insoluble silver gelatinate of definite proportions formed in amounts varying with the conditions, as it would require that with constant total silver the combined silver per gram of gelatin should increase as the gelatin concentration decreased." There appears to be a confusion of ideas, or a lack of adequate expression, in this statement. In the first place, while it is true (as the present work confirms) that at constant pH and free silver ion concentration ($[Ag^+]$), the extent of combination with silver is independent of the gelatin concentration, it does not follow from this that with constant <u>total</u> silver the combined silver cannot increase with decrease in the gelatin concentration - actually, it does increase. In the second place, increase in the combination with decrease in gelatin

concentration, at constant total silver, does not necessarily signify the formation of an insoluble silver salt, but will be the case for any type of combination.

Pauli[1] holds that the negatively-charged groups, i.e., the carboxyl groups, of the protein are responsible for the decrease in the activity of the silver ions. The fall in activity is considered as due either to "inactivation" (presumably through ionic interaction of the Debye-Hückel type) of the silver ions, or, in more extreme instances, to actual combination of the silver ions with the carboxyl groups of the protein. Pauli also postulates that the positive groups of the protein molecule counteract the influence of the carboxyl groups upon the activity of the silver ions. This view is based upon the work of Goigner and Pauli (loc. cit.), whose experiments with ovalbumin, serum albumin and gelatin appear to show that the order of decreasing influence upon the activity of the silver ions, is also the order in which the ratio of positive groups to negative groups increases. Again, Pauli attributes the greater decrease in activity of the silver ions in the case of silver hydroxide, as compared with silver nitrate, to neutralisation of the positive groups. It is evident that, on Pauli's hypothesis, deamination of gelatin should produce an increase in the amount of

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(1) Pauli, Kolloid-Zeitschr., 53 (1930) 51.

combined silver. Actually, however, as the present investigation shows, there is, if anything, a slight decrease.

Assuming that the silver is attached at the carboxyl groups, it might be thought that the increase in combination with increase in pH could be traced to increase in the number of carboxyl groups as the result of hydrolysis of the acid amide groups, or of the peptide linkages.(1) From the available analytical data(2) it would appear that the free carboxyl groups in gelatin amount to about 0·42 m.e./g., and the acid amide groups to about 0·24 m.e./g. Since the carboxyl groups are in all probability fully ionised at pH = 9·0, the number of COO^- groups can, therefore, increase by only about 50% by hydrolysis of the amide groups. This is obviously quite inadequate to account for the six-fold increase in combined silver which occurs between pH = 9·0 and pH = 10·5. Moreover, the alkali bound between pH = 9·0 and pH = 10·5 is only about the same as that bound between 4·7 and 9·0, and part, at least, of the additional alkali must be neutralised by NH_3^+ groups. The conclusion, therefore, is that the

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(1) That some hydrolysis may occur under the given conditions is suggested by the observation of Pleass (Biochem. J., 24 (1930) 1472) that the pH of a one per cent. solution of gelatin, if greater than 6·5, decreases considerably during one week, carbon dioxide being carefully excluded. See also Jordan Lloyd and Shore (loc. cit.) p. 312.

(2) See Jordan Lloyd and Shore (loc. cit.), Table VII, p. 76; Schmidt (loc. cit.) pp. 733, 734.

formation of COO^- groups by hydrolysis of the protein takes place on far too small a scale to account for the increase in combined silver between pH = 9·0 and pH = 10·5. Thus while the possibility of combination of silver ions at the COO^- groups cannot, at present, be ruled out altogether, the existence of some other type of combination is strongly indicated by the results of the present work.

We may now consider the influence of silver nitrate upon the electro-endosmotic flow in gelatin gels. As is shown by the experimental data in Tables IX (a)-(e) (pp. 49 - 53), it was observed that the rate of flow was decreased by the addition of the silver salt, except in the case of deaminated gelatin at pH = 10·5 (Table IX (e)), where no effect was produced. Moreover, the rate of flow is directly proportional to the difference between the combined alkali and the combined silver. The values of this difference are given in the fifth column of Table XVIII (p. 105), and the last column of the table contains the values obtained by multiplying the rate of flow by 0·033, which was the factor used in the interpretation of the influence of alkali upon the rate of flow (p. 85). The value for the rate of flow for purified gelatin at pH = 9·0 has been corrected by the addition of 0·397 - 0·297 = 0·100, to allow for the fact that in the absence of silver nitrate the rate of flow at pH = 9·0 was too low (see Table XIV, p. 86).

TABLE XVIII.

Gelatin	pH	Combined alkali (m.e./gm)	Combined silver (m.e./gm)	Difference (m.e./gm)	Calculated from electro-endosmosis (m.e./gm.)
Purified	7.0	0.282	0.061	0.221	0.201
	9.0	0.397	0.082	0.315	0.185*
	10.5	0.815	0.358	0.457	0.449
Deaminated	7.0	0.679	0.051	0.628	0.673
	9.0	(0.797)	(0.071)	(0.726)	-
	10.5	1.243	0.354	0.889	1.226

*Corrected value = 0.185 + (0.397 - 0.297) = 0.285.

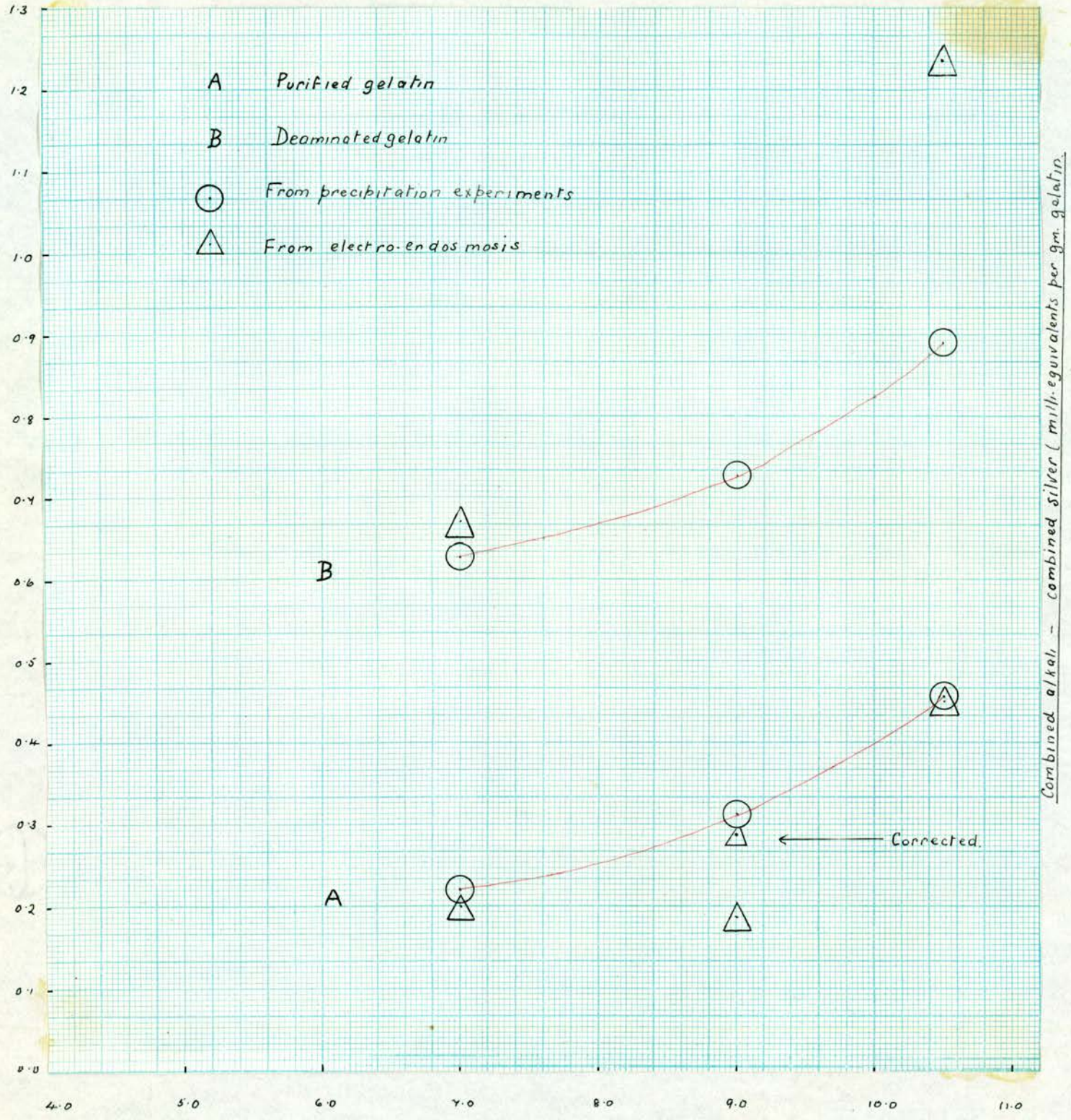
A Purified gelatin
B Deaminated gelatin
From precipitation experiments
From electro-endosmosis
B
A
Corrected.
1·3
1·2
1·1
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0·0
4·0
5·0
6·0
7·0
8·0
9·0
10·0
11·0
pH
Combined alkali - combined silver (milli-equivalents per gm. gelatin
Combined alkali - combined silver (milli-equivalents per gm. gelatin

FIGURE XII

It will be seen from Table XVIII, and also from Figure XII, in which the derived values are plotted, that the product "rate of flow × 0·033" is equal to the difference "combined alkali - combined silver" (except in the one case mentioned above). From this result it is reasonable to conclude that for undeaminated gelatin over the pH range 7·0 - 10·5, and for deaminated gelatin at pH = 7·0, (a) the precipitation method gives a reliable estimate of the amount of combined silver, (b) at constant ionic strength the rate of electro-endosmotic flow is directly proportional to the charge on the protein molecule, and (c) the silver combines with the protein in the form of silver ions, i.e., the gelatin does not unite with molecules of silver nitrate to form some kind of molecular compound.

At present it is difficult to interpret the data for deaminated gelatin at pH = 10·5, since the amount of combined silver is the same as for undeaminated gelatin, and yet the rate of flow remains the same as in the absence of silver nitrate. The electro-endosmotic experiments were carried out with the usual degree of care, and it would therefore appear that the silver is combined in some form other than that of simple ions. It is not easy to account for this state of affairs.

The final data obtained for the electrophoresis

TABLE XIX.

Electrophoretic Mobility.

pH	Conc. $AgNO_3$ (m.e./l.)	Ionic strength	Observed time (secs)	Mobility (μ/sec/volt/cm)	Mobility × 16.73 *
7.0	-	1.18	7.2	0.702	11.74
"	2.15	3.33	8.2	0.615	10.29
"	-	6.43	8.8	0.574	9.60
	0.74	6.42	9.5	0.532	8.90
"	-	15.50	9.6	0.526	8.80
	14.32	15.50	10.4	0.485	8.11
10.5	-	4.27	5.8	0.870	14.55
"	2.15	6.42	6.3	0.801	13.40
Ca.4.0	-	4.00	9.8	(+) 0.516	(+)8.63

$$* \text{ Factor} = \frac{\text{Rate endosmotic flow at pH = 7·0 and ionic strength = 23·66}}{\text{Electrophoretic velocity at pH = 7·0 and ionic strength = 15·50}}$$

of silica particles suspended in gelatin solutions are collected in Table XIX (p. 108). This work was of an exploratory nature, and purified gelatin only was investigated. It will be observed that the presence of silver nitrate had no appreciable effect upon the electrophoretic velocity of the particles. The small differences obtained in the experiments in which 2·15 m.e./l. silver nitrate was used may be attributed to differences in the ionic strength. In view of the well-marked influence of silver nitrate upon the electro-endosmotic flow, the absence of effect in the case of the electrophoretic experiments is surprising. There appear to be no definite grounds for ascribing the discrepancy to faulty technique in the measurement of the mobility. When the pH was on the alkaline side of the iso-electric point, the particles moved to the anode, and the movement was reversed when the pH was brought to the acid side. It was also observed that when the gelatin was used without the addition of acid or alkali, there was no appreciable movement of the particles. Moreover, increase in the pH, from 7·0 to 10·5, resulted in a considerable increase in the electrophoretic velocity, although the change was not proportional to the change in rate of electro-endosmotic flow, as the last column in Table XIX shows.

Rideal and Wright[1] state that the adsorption of

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(1) Rideal and Wright, Trans. Faraday Soc., 24 (1928) 539.

silver (from solutions of $AgNO_3$) by silica is very small, but is considerably increased on addition of a trace of alkali. It therefore appears just possible that, in the present experiments, the silver combined with the gelatin coating the particles of silica is also adsorbed by the silica, and hence is screened by the charged groups of the protein. This implies that the silver is not attached to any of the charged groups in the gelatin molecule.

Kruyt and Boelman[1] found (a) that for a given concentration of free silver ion, the amount of silver "bound" by unit quantity of albumin was independent of the concentration of albumin, and (b) that the amount of silver "bound" by unit quantity of gelatin was less when the solution of gelatin was prepared from material which had been dried by exposure to a stream of hot air, than when the gelatin was not subjected to such treatment. It is deduced that the binding of the silver must be a surface phenomenon, and the silver is considered to be "adsorbed" by the protein. The nature of the "adsorption" is not definitely specified, but since the data are shown to fit equations of the well-known Freundlich type fairly well, it is to be assumed that Kruyt and Boelman have in mind an effect, due to secondary valencies, in which the silver ions are not located at definite positions in the protein molecule, but distributed statistically over the "surface" of the

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(1) Kruyt and Boelman, Kolloid-Beih., 35 (1932) 165.

micelle.

Actually, the experimental results put forward by Kruyt and Boelman as evidence that the silver is "adsorbed", can be equally well interpreted on the basis of chemical combination. In the first place, it can be deduced from the mass law that the amount of chemically combined silver per unit mass of protein will be constant when the concentration of free silver is constant, irrespective of the concentration of the protein. Secondly, it is as reasonable to suppose that the decrease in bound silver which results from the drying of gelatin is due to some change which affects the combining capacity of the protein, as to postulate a decrease in the available surface area.

The data obtained in the present investigation were found to be fairly well represented by Freundlich's isotherm, in agreement with Kruyt and Boelman, but the significance of this relation still remains obscure. The e.m.f. curve (Fig. V, p. 65) is also difficult to interpret.

Langmuir's equation in its simplest form, i.e., for the case of adsorption at a limited number of points of one kind on a surface[1], was found to be inapplicable to the present data. It would therefore appear that that the silver is bound by more than one type of group in the gelatin molecule, and this is also suggested by

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(1) As Hitchcock (J.A.C.S., 48 (1926) 2870) has pointed out, it is at present impossible to distinguish between mass law combination and adsorption which obeys Langmuir's equation.

the manner in which the amount of combined silver varies with the pH.

Hydrolysis of the gelatin was found to decrease the amount of combined silver (see Fig. IX, p. 75). Coigner and Pauli (loc. cit.) also observed a considerable change in the same direction with purified gelatin which had been heated (2·5 per cent. solution in autoclave) at 125° for one hour. It would therefore seem that the decrease in combination which resulted from purification of the stock gelatin was not due to the removal of decomposition products produced by simple thermo-hydrolysis.

In conclusion it may be pointed out that the combination of silver with amino-acids has received little attention, although investigations in this field might throw light on the combination with proteins. Coigner and Pauli carried out a few experiments with glycine and alanine, but their data are too limited to show more than that combination probably occurs.

S U M M A R Y.

1. Determinations have been made of the concentration of gelatin required to prevent the separation of the solid phase from aqueous mixtures of silver nitrate and potassium chromate at 25°.

2. It has been found that under the given conditions of constant concentration of potassium chromate, constant pH, and constant period of observation, the concentration of gelatin increases with the concentration of silver nitrate in a characteristic manner.

3. The influence of the following factors upon the precipitation curve has been investigated:-
(a) concentration of potassium chromate, (b) pH,
(c) purification, hydrolysis, and deamination of the gelatin.

4. By means of the silver electrode, determinations have been made of the activity of the silver ion in aqueous mixtures of gelatin and silver nitrate at 25°, and pH = 7·0.

5. The influence of alkali and of silver nitrate upon the electro-endosmotic flow in gels of ordinary and of deaminated gelatin has been investigated.

6./

6. The influence of alkali and of silver nitrate upon the electrophoresis of silica particles suspended in gelatin solutions has been examined.

7. It has been confirmed that gelatin opposes the formation of solid silver chromate from supersaturated solutions of the salt, and it has been found that:

(a) With increase in the concentration of gelatin, the degree of supersaturation at first increases, but eventually becomes constant.

(b) The maximum degree of supersaturation is independent of the pH over the range 7·0 to 10·5.

(c) The maximum degree of supersaturation is not affected by purification, hydrolysis, or deamination of the gelatin.

(d) Hydrolysis of the gelatin appears to decrease the concentration of the protein at which the maximum degree of supersaturation is attained.

(e) The concentration of gelatin at which the maximum degree of supersaturation is attained is very much higher than values corresponding to the gold numbers which are commonly obtained for gelatin.

8./

8. It has been deduced from the precipitation and e.m.f. data that the reduced activity of the silver ion in solutions of silver nitrate containing gelatin must be due to combination of the silver with the protein, and that interionic action is of very minor importance under the given conditions.

9. It has been shown that the precipitation curve provides an accurate means of determining the amount of silver combined with the gelatin, of particular use at relatively high pH values, where the silver electrode was found to be unreliable.

10. The following relation has been found to hold for the potential of the silver electrode in solutions of constant silver, but varying gelatin content:-

Potential in gelatin solution = potential in absence of gelatin + constant × concentration of gelatin.

11. It has been shown that the amount of silver bound by unit mass of gelatin is:-

(a) constant at constant pH and constant concentration of free silver ions, irrespective of the concentration of gelatin;

(b) increased with increase in pH, the effect being small between 7·0 and 9·0, but very marked between 9·0 and 10·5;

(c) unaffected, or slightly decreased, by deamination of the gelatin;

(d) decreased by purification or hydrolysis of the gelatin;

(e) obeys the ordinary adsorption isotherm fairly closely;

(f) does not obey Langmuir's adsorption isotherm in its simplest form.

12. It has been found that at a given pH and ionic strength, the rate of electroendosmotic flow through gelatin gels is (a) directly proportional to the amount of combined alkali, in the absence of silver nitrate, and (b) directly proportional to the difference between the combined alkali and the combined silver, except in the case of deaminated gelatin at high pH values.

13. It has been observed that silver nitrate has no influence upon the electrophoretic mobility of particles of silica suspended in solutions of gelatin.

14. It is concluded that:-

(a) over the range of pH 7·0 - 10·5, the rate of electro-endosmotic flow in gelatin is, at constant ionic strength, directly proportional to the net charge on the protein molecules;

(b) in aqueous mixtures of gelatin and silver nitrate, the protein combines with simple silver ions, except in the case of deaminated gelatin at high pH values;

(c) the silver does not combine with the free amino groups of the gelatin;

(d) if combination of the silver with the free carboxyl groups of the gelatin occurs, only part of the silver is so combined;

(e) it is probable that the silver is combined with the gelatin in more than one fashion.

In conclusion the author wishes to express his appreciation of the unfailing help and guidance given by Dr T. R. Bolam throughout the course of this research.